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In year one of the current project, experiments have been performed to:

**1) Examine the "normal" progression of SIV infection and disease using standardized conditions and the various assays proposed for examination of animals receiving DNA vaccines (including virus load, cellular and humoral immune responses, and cytokine profiles).** Standatdization of our SIV macaque animal model system was performed in preparation for genetic immunization versus SIV immunization. Assays for viral load, antiviral and cellular immune responses and cytokine levels (to SIV and nonspecific antigens) were applied to four SIV infected and two uninfected rhesus macaques. Considerable innate individual animal variation was observed in immune responsiveness to SIV and other antigens, as was corresponding variation in ability to control SIV infection. Our laboratory assays also revealed, somewhat unexpectedly, problems which now largely been resolved.

**2) Develop novel expression vectors intended to improve levels of expression of SIV proteins from DNA injection in mice and primates.** A number of novel expression vectors have been designed and tested in cell culture and in animals. These expression vector constructs were found to be very active relative to previously available constructions, and are now being adapted for the expression of a variety of SIV envelope proteins.

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Murray B. Gardner

Murray B. Gardner, M.D.
Principal Investigator

8/30/95
Date

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## Title Page

## Grant # DAMD17-94-J-4436
## "Genetic Immunization for Lentiviral Immunodeficiency Virus Infection and Disease"


| section | page numbers |
|---|---|
| **Introduction** | **5-6** |
| **Body** | **6-22** |
| **1) Examination of "normal" progression of SIV.** | **6-15** |
| **Methods** | **6-9** |
| **Results** | |
| **Executive summary** | **9** |
| **Infection and viral load** | **9-10** |
| **Humoral immune responses** | **10-11** |
| **Cellular immune responses** | **11-12** |
| **Cytokine profiles** | **12** |
| **Conclusions** | **12-15** |
| **2) Development of novel expression vectors** | **16-22** |
| **Vector Design** | **16-17** |
| **Vector Construction** | **17-18** |
| **In vitro and in vivo testing** | **18-21** |
| **Conclusions** | **21-22** |
| **Revised research plan for year 2** | **22-25** |
| **References** | **25-27** |
| **Appendicies** | |
| **Appendix 1: Results of cytokine analyses** | |
| **Appendix 2: Results of cellular and humoral assays** | |
| **Appendix 3: Revised budget, Emory Subcontract** | |
| **Appendix 4: Memorandum from California Primate Research Center** | |

## Introduction

Genetic immunization (DNA vaccination) and cytokine augmentation of antiviral immune responses are novel areas of vaccine research. Limited studies which have been performed by ourselves and other investigators support the notion that plasmids expressing viral genes induce protective antiviral immune responses in small animals, and that exogenously administered cytokines can also influence pre-existing or developing host immune responses. However, the efficacy of genetic vaccines (with or without cytokines and/or activation signals such as B7) directed against lentiviruses which cause fatal immunodeficiency in primates (including humans) has not been methodically evaluated. In addition, we have yet to define immune responses in HIV or SIV vaccinated primates that do confer protection versus HIV or SIV versus those that do not. This proposal is aimed at defining a "safe" genetic immunization protocol capable of inducing protective immunity versus SIV and concurrently identify the immune mechanisms responsible for this protective immunity. The identification of these mechanisms is expected to further open the way to effective post exposure immunotherapies for already infected individuals.

The immune response in mice after nucleic acid vaccination is characteristically long lived. A single intramuscular injection of 20 mg of DNA containing the influenza NP gene produces an IgG antibody response which persists for more than two years. A similar kinetic pattern has been observed for almost a dozen antigens including gp120 from HIV-1. In contrast, the immune response to gp120/130-encoding DNA from HIV-1 or SIV observed in primates has been weak and transient. Similar results have also been obtained using the gene gun for vaccination. These primate results are difficult to compare to the mouse data because both the antigen and the species have changed. Thus, there are (at least) two possible explanations for these results;

1) The weak and transient response could be because *injection conditions are not optimized for primates* resulting in low antigen expression and a weak immune response. Some experimental data in rodents which make such a hypothesis plausible. If mice and rats are injected intramuscularly under identical conditions (50 mg of DNA in 50 ml of solution), expression is found to be at least 5 fold higher in mice. However, equal expression can be obtained in both species by simply increasing the injection volume in the rat to 300 ml. This is most likely related to the size of the muscle as the rat quadracepts is approximately 10 fold larger than the mouse. The revised research plan proposes investigating the effect on the immune response in primates of the amount of DNA injected and the volume of injection.

2) Alternatively, *the gp160 protein may contain elements which suppress the immune response*. It is almost a tautology to suggest that a virus which establishes a chronic infection in a host has mechanisms to evade the host immunity. The above results suggest that gp160 may contain regions in the C-terminal portion which actively suppress immunity to the molecule. Several immunogenic domains in the gp41 region which have been defined by synthetic peptides, and we propose to include two additional plasmids represneting these domains in the initial screen for immunogenicity. These plasmids contain all of the gp130 region and part of gp41. Recently, researchers have documented that the carboxyterminal 42 amino acids of gp160 are cytotoxic (see below). Hence, we will delete this portion of gp160, which may be immunosupressive.

The simian immunodeficiency virus from macaque (SIVmac) is a primate lentivirus with approximately 45% genetic homology to HIV I and 75% homology to HIV II (1, 2, 3). Like HIV, SIV is lymphocytotrophic and uses the CD4 protein as its cellular receptor (4) . The disease induced by SIVmac in macaques closely resembles human AIDS, with terminal

death within 12-36 months (5, 6, 7, 8, 9, 10, 11). The biologic, pathologic, and genetic similarities between HIV infection in humans and SIVmac infection in macaques, combined with the abbreviated clinical course, makes SIVmac/rhesus macaque an excellent model for developing and studying HIV vaccination strategies. This project utilizes this model prospectively to demonstrate the feasibility and efficacy of genetic immunization to elicit a protective immunity for lentivirus induced disease in primates.

In year one of the current project, experiments have been performed to:

**1) Examine the "normal" progression of SIV infection and disease using standardized conditions and the various assays proposed for examination of animals receiving DNA vaccines (including virus load, cellular and humoral immune responses, and cytokine profiles),**

**2) Develop novel expression vectors intended to improve levels of expression of SIV proteins from DNA injection in mice and primates, and**

This report will detail the results of this experimentation.

The results of two SIV challenge experiments in macaques utilizing SIV nucleic acid vaccination have been reported in the last few months. The group lead by H. Robinson showed no protection of animals challenged with SIV after repeated vaccination with plasmid DNA expressing gp160. The group lead by D. Weiner has vaccinated animals with plasmids containing the HIV-1 *rev*, *tat*, *env* and *nef* genes. Challenge in this case was with SHIV (HIV envelope) hybrid virus and two animals (less than 50%) appeared to be protected. These disappointing results lead us to reconsider the second year research plan for this grant, and therefore this report also includes plans for modification of the grant aims and experimentation.

## Body

### 1) Examination of the "normal" progression of SIV infection and disease using standardized conditions and the various assays proposed for examination of animals receiving DNA vaccines (including virus load, cellular and humoral immune responses, and cytokine profiles).

The main objective of this project for the first year was to measure (i) levels of virus, (ii) immune responses to viral (SIV) and control (tetanus toxoid, influenza, KLH) antigens, and (iii) measure levels of several cytokines in rhesus macaques infected with uncloned pathogenic SIVmac251. These studies are intended to provide data for correlating cytokine levels with potentially protective anti-viral immune responses.

**Methods:**

Inoculation of rhesus macaques: All animals were colony-bred juvenile rhesus macaques (*M. mulatta*) free of SRV, SIV, and STLV; these animals were housed at the California Regional Primate Research Center (CRPRC) at Davis, CA in accordance with American Association for Accreditation of Laboratory Animal Care Standards. Before inoculation, 20 ml of blood was collected by venipuncture for plasma, complete blood count (CBC) including platelet count, and CD4/CD8 T lymphocyte phenotyping by flow cytometry. Peripheral lymph nodes were obtained by excisional biopsy, and portions of

lymph nodes were fixed in formaldehyde and OCT. Animals were observed daily and weighed once weekly by the CRPRC veterinary staff. Complete physical examinations were performed before and after inoculation to detect weight loss, lymphadenopathy and/or splenomegaly, and opportunistic infections. A stock of uncloned SIVmac251, titered for macaque infectious doses (MID), was obtained from Dr. Ron Desrosiers (New England Regional Primate Research Center). Each of four juvenile macaques were inoculated by the intravenous route with 100 MID50 of this virus. Two uninfected macaques were entered into this study as uninfected control animals.

Sample collections for measuring in vivo viral loads: 10 to 20 ml of peripheral blood was collected in heparanized tubes and plasma and peripheral blood mononuclear cells (PBMCs) were separated by centrifugation in a Ficoll/Hypaque density gradient. Peripheral lymph nodes were collected by excisional biopsy.

Titration of virus in plasma, PBMC, and LNC: Levels of viral antigen in plasma were measured with an ELISA kit for SIV $p27^{gag}$ antigen (Coulter Immunology, Hialeah, FL). For measuring plasma viremia, serial 10-fold plasma dilutions were made in tissue culture medium and dispensed into 24-well microtiter plates containing 2.5 X $10^5$ CEMX174 cells. Growth of virus was scored by microscopic observation of cytopathology and by measuring viral antigen in tissue culture supernatant with the SIV $p27^{gag}$ ELISA kit. For measuring virus in PBMCs, $10^6$ PBMC (and serial 10-fold dilutions of PBMC) from each infected macaque were co-cultured with 2.5 X $10^5$ CEMX174 cells per well with 4 wells per dilution . Samples of media from these co-cultures were observed for cytopathology and assayed for SIV $p27^{gag}$ antigen by the ELISA kit to monitor virus production. To measure virus load in lymph nodes, peripheral lymph nodes were obtained by transcutaneous biopsy and aseptically teased into single-cell suspensions; cell numbers were determined by counting in a hemocytometer. Ten-fold dilutions of lymph node cells were co-cultured with CEMX174 cells. Titers were calculated by the method of Reed and Meunch.

ELISA assay for determination of humoral response. Enzyme-linked immunosorbent assay (ELISA) were used to measure the humoral responses of monkeys. Immunoreactivity with viral antigens and recognition of whole virus lysates has been described previously (12).

Antigen-specific IgA quantitation using peripheral blood mononuclear cells (PBMC). No one method has yet been generally accepted as the most sensitive, reproducible method for determining antigen-specific Immunoglobulin A (IgA) immune responses. Fecal samples, vaginal washes, serum, colostrum, saliva, and circulating mononuclear cells (PBMC) have been used to determine levels of specific IgA antibody (13, 14, 15). Recently, vaginal washes have been relied upon extensively by groups attempting to quantitate genetal mucosal immune responses (15, 16, 17). C. Miller *et al.* indicate, however, that IgA antibodies often detected in vaginal washes are likely antibodies that have simply leaked into the mucosa from the serum (18). As a result, this assessment might not represent an accurate assessment of a locally derived mucosal immune response. However, various IgA sample sources, including intestinal fluid, saliva, circulating PBMC, and serum, were recently compared to determine which gave the most sensitive and correlative indicator of a specific intestinal-IgA immune response (13). IgA production by PBMC was found to be the best indicator of a specific mucosal response. We have adapted this assay for use with both rhesus macaques and mice to monitor any mucosal response after infection or immunization with the vaccine constructs. Peripheral blood mononuclear cells are isolated by Ficoll gradient purification. PBMC are then be washed 3x in RPMI 1640, and

resuspended in 10% FCS-supplemented RPMI 1640 to a concentration of $5x10^6$ PBL/ml. Recombinant gp130 expressed in CHO cells and produced in our laboratory, is plated at 200ng/well, 50ul/well in a 96-well standard polystyrene ELISA plate (Fisher, Pittsburgh, PA). Plates are then incubated overnight at 37°C. Plates are washed ten times with PBS containing 0.1% tween-20, and 5% Blotto (nonfat milk protein) at 100ul/well and left to block at 37°C for one hour. After ten washes, 100ul ($5x10^5$ PBMC/well) of the resuspended PBMC are added to each well and left to incubate at 37°C, 5% $CO_2$ for 16 hours. After ten washes, rabbit anti-monkey IgA and IgG antibody (Nordic Immunological Laboratories, Capistrano Beach, CA) or rabbit anti-mouse IgA and IgG (Fisher, Pittsburgh, PA) have been added at a 1:1000 dilution, 50ul/well. Plates are left to incubate for 4 hours at 37°C. After washing, goat anti-rabbit-labeled alkaline phosphatase (Fisher, Pittsburgh, PA) is added at 1:1000, 50ul/well, and incubated for 1 hour at 37°C. Finally, 100ul/well of 1mg/ml PNP substrate (Zymed Laboratories, San Francisco, CA) is added to the plates, and plates are read at 405nm. ELISA plates with no PBMC added are used as blanks, and PBMC from unimmunized monkeys or mice serve as negative controls.

Anti-viral CTL activity. SIV antigen-specific secondary CTL activity is measured by a conventional $^{51}$-chromium-release assay after antigen-specific stimulation in vitro of PBMC or lymph node lymphocytes (LNL). To prepare stimulator cells, autologous CD4+ T cells (negatively selected with anti-CD8 magnetic beads, Dynal) are infected with SIVmac239 and stimulated with staphylococcal enterotoxin A, 0.5 μg/ml, for 5 days in medium supplemented with 50 U/ml recombinant human IL-2. On day 4 of culture, the medium is exchanged without supplemented IL-2, and on day 5, the cells are washed twice and counted prior to addition to fresh PBMC or LNL in co-culture. These co-cultures are started with a stimulator/responder ratio of 10:1 at 2 X $10^6$ cells/ml in RPMI 1640 medium/10% FCS without supplements. After 48 hours, conditioned medium from human PBMC stimulated with PHA (Schiapparelli Inc.) is added to 5%, and after 7 days, human recombinant IL-2, 20 U/ml, is added to stimulate cell growth. The cultures are harvested for effector cells between days 12 and 16 after initiation. In some experiments, to quantify CTL activity by precursor frequency analysis, the co-cultures are established by limit dilution in 96-well round bottom plates, with irradiated human PBMC as feeder cells (19). For the CTL assay, autologous B lymphocytes are transformed by *H. papio* (20), and infected overnight with recombinant vaccinia viruses expressing SIV-specific genes (Pr55gag, pol, gp160 or nef). Vaccinia virus without SIV inserts is the control. Effector and target cells are added at multiple E/T ratios in a 4 hour chromium-release assay, and percent specific lysis is calculated from supernatant chromium measured with a beta counter (Wallac micro-beta). For precursor frequency analysis, uncounted culture wells are split into 3 wells of the assay plate, and lysis of the SIV gag or gp160 targets are scored against lysis of the wild-type vaccinia target by 3 standard deviations. Precursor frequency is calculated as the number of CTL per $10^6$ starting lymphocytes by Poisson distribution (19). The phenotype of effector cells is determined by depletion of CD4 or CD8+ T cells using antibodies conjugated to magnetic beads (Dynal Inc.), and MHC restriction is determined by comparing lysis of the murine cell line, P815, expressing SIV antigens.

Analysis of cytokine mRNA levels: Four rhesus macaques were infected with uncloned SIVmac 251 as described above, and at varying time intervals, blood was collected in heparin. PBMC were isolated, and an aliquot was washed, pelleted, and snap-frozen, whereas another aliquot was cultured with PHA-P (2 mg/ml) for approximately 24 hrs,

centrifuged, washed, and the pellet was snap-frozen. Similar samples, handled in the same way, from two non-infected rhesus macaques were also prepared.

The unstimulated (ex vivo) and the PHA-P-stimulated PBMC were sent to the laboratory of Dr. A.A. Ansari at Emory University for analysis. RNA was extracted from each specimen and subjected to semi-quantitative RT-PCR analysis for: IL-1b, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-g, TNF-a, and GAPDH (housekeeping).

**Results:**

Executive summary

The first year of this grant was dedicated to standardizing our SIV macaque animal model system in preparation for genetic immunization versus SIV immunization. Assays for viral load, antiviral and cellular immune responses and cytokine levels (to SIV and nonspecific antigens) were applied to four SIV infected and two uninfected rhesus macaques. This "shakedown cruise" revealed, as expected, considerable innate individual animal variation in immune responsiveness to SIV and other antigens, and corresponding variation in ability to control SIV infection. Our laboratory assays also revealed, somewhat unexpectedly, problems which now largely been resolved. An appreciation of such animal to animal variables and vagaries in assay conditions are essential for our ability to evaluate and understand the positive or negative results of genetic immunization versus SIV.

Despite the promising immunogenicity and efficacy results of genetic immunization against certain infectious agents in small animals, equally encouraging results have yet to be realized in non-human primates against any pathogen, much less SIV. During this last year, two other laboratories largely failed to protect macaques from SIV challenge infection by genetic immunization with SIVenv encoding DNA. Therefore, before our challenge experiments are performed, we will do more preparatory work by:

1) Determining the optimal doses, volumes, and routes of DNA inoculation in mice and macaques in order to maximize humoral and cellular responses.
2) Preparation of the most ideal expression vector for presentation of selected SIV envelope sequences.

Since we only have the funding to perform one limited efficacy test, we wish to get the necessary techniques and reagents assembled and evaluated before challenge, so that we will have the best chance for success.

Infection and Viral Load analyses.

Values for plasma viremia are shown in Table 1, and cell-associated virus loads in PBMCs and LNCs are presented in Tables 2 and 3, respectively. All four macaques exhibited moderate to high virus loads during the acute stage of infection (0 to 4 weeks). After this initial phase, two macaques (Mmu26161 and Mmu26230) maintained relatively high amounts of virus and infected cells, whereas two macaques (Mmu26470 and Mmu26474) displayed relatively low virus loads. Mmu26161 exhibited signs of simian AIDS (SAIDS), including decline of both CD4 cell numbers and CD4/CD8 ratios, and was euthanized at 20 weeks post-infection. Pathological and histopathological analysis at necropsy confirmed the diagnosis of SAIDS. The other three macaques were euthanized at

26 weeks post-infection. Numerous necropsy specimens have been collected and preserved for continued studies.

**Table 1: Levels of plasma virus in macaques infected withSIVmac251 ($TCID_{50}$/ml plasma).**

| Weeks PI | 2 | 4 | 6 | 8 | 12 | 16 | 20 |
|---|---|---|---|---|---|---|---|
| Macaque | | | | | | | |
| Mmu26161 | 100,000 | 316 | 100 | 3,162 | 10,000 | 100 | 4,642 |
| Mmu26230 | 100,000 | 1,000 | 22 | 100 | 316 | 10,000 | 100 |
| Mmu26470 | 10,000 | 32 | 100 | 46 | 316 | 100 | < 1 |
| Mmu26474 | 100,000 | 100 | 10 | < 1 | < 1 | 10 | < 1 |

**Table 2: Levels of cell-associated virus loads in peripheral blood mononuclear cells in macaques infected withSIVmac251 ($TCID_{50}$/million PBMC).**

| Weeks PI | 2 | 4 | 6 | 8 | 12 | 16 | 20 |
|---|---|---|---|---|---|---|---|
| Macaque | | | | | | | |
| Mmu26161 | 10,000 | 100 | 1,000 | 2,154 | 464 | 46 | 3,162 |
| Mmu26230 | 3,162 | 3,162 | 215 | 316 | 1,000 | 316 | 464 |
| Mmu26470 | 1,000 | 464 | 1,000 | 464 | 316 | 100 | 32 |
| Mmu26474 | 10,000 | 316 | 32 | 32 | 464 | 46 | 22 |

**Table 3: Levels of cell-associated virus loads in lymph node mononuclear cells in macaques infected withSIVmac251($TCID_{50}$/million LNMC).**

| Weeks PI | 2 | 12 | 20 |
|---|---|---|---|
| Macaque | | | |
| Mmu26161 | 316 | 1,000 | 1,000 |
| Mmu26230 | 10,000 | 1,000 | ND |
| Mmu26470 | 1,000 | 100 | ND |
| Mmu26474 | 3,162 | 464 | ND |

Analyses of Humoral Immune Responses.

The negative control animals (26313 and 26438) were immunized with KLH and tetanus toxoid, but not challenged with SIV. These animals and the four animals challenged with SIV showed appropriate responses to both KLH and tetanus toxoid. The two control animals showed no reactivity against SIV, as determined in the whole virus ELISA assay. Of the four animals challenged with live SIV on February 7, 1995, animals 26161 and 26470 showed no reactivity against intact SIV in the ELISA assay. These two animals appeared to be non-responders even though we confirmed that they were infected with SIV.

Animal 26161 showed SAIDS symptoms relatively early and deteriorated rapidly in the absence of a measurable humoral immune response against SIV.

For this part of the project, T cell proliferation was done with intact proteins (tetanus toxoid, KLH and rgp130). We observed proliferative responses to KLH and tetanus toxoid in animals 26161, 26313, 26438 and 26320 before challenge with live SIV. Animals 26474 and 26470 did not show significant T cell proliferation values before challenge. Following challenge with SIV, we detected significant proliferation (SI) against KLH in all the animals, although the control animal 26438 did not develop a proliferative response to KLH for an unknown reason. Tetanus toxoid SI were significant for three of the animals, but negative in animals 26438, 26161, and 26320. Proliferation values to rgp130 were insignificant in all SIV infected and uninfected control macaques.

In the macaque PBMC, IgA and IgG secretion assay we were able to detect the production of both classes of antibody against SIV in animals 26470 and 26474, but no response was detected in animal 26161 and 26320. Tetanus Toxoid antibody levels were high in all the animals but KLH reactivity was very low.

Analyses of Cellular Immune Responses.

SIV-specific CTL responses were assayed at monthly intervals in the four macaques infected with SIVmac251. The results overall were unexpectedly poor and inconsistent from month to month for each of the animals. Monkeys infected with pathogenic strains of SIVmac generally have low to moderate CTL responses to SIV antigens that are detectable after stimulation of their PBMC with the mitogen, ConA. As shown in the Table below, this pattern of response was not seen. In the beginning of the study we had problems with the assay: our commercial source of RPMI 1640 medium was found to cause stimulation of lymphocyte cultures, and we had to screen other commercial sources of RPMI. However, the assay performed at week 16 was clearly negative for all 4 animals, without obvious problems with the assay itself. At necropsy, CTL responses to gag and env were readily detected in PBMC and spleen or peripheral lymph node from two of four animals. The monkey that developed simian AIDS, 26161, had no detectable CTL response to env at week 16 or at necropsy.

**Table 4: SIV-specific CTL responses of 4 rhesus monkeys infected with SIVmac251**

| monkey | week 4 | week 8 | week 12 | week 16 | week 20 necropsy | week 24 necropsy |
|---|---|---|---|---|---|---|
| | gag/env | gag/env | gag/env | gag/env | gag/env | gag/env |
| 26161 | ind / neg | neg / 45% | ind / ind | neg / neg | nd / neg | |
| 26320 | neg / neg | neg / 150% | neg / neg | neg / neg | | 36%/22% |
| 26470 | nd | 11% / 14% | neg / neg | neg / neg | | neg/neg |
| 26474 | nd | nd | 32% / 12% | neg / neg | | 50%/46% |

| | |
|---|---|
| gag/env | maximum specific lysis of target cells expressing p55$^{gag}$ or gp160$^{env}$ antigens |
| ind | indeterminant lysis due to high lysis of negative control targets |
| nd | not done |
| neg | negative |

Analysis of Cytokine Profiles:

The relative number of copies of mRNA for each of the cytokines was calculated as described elsewhere {1,2}. Data analysis was performed and is graphically illustrated in Figs. 1, 2A, and 2B. Fig. 1 graphically illustrates the data from the two control monkeys 26313 and 26438. Figs. 2A and 2B illustrate the data from the four SIV-infected monkeys (26161, 263620, 26470, and 26474). The data obtained are summarized below:

(i) No detectable or reproducible levels of IL-2 or IL-12 message were noted. However, controls also were a problem. Aliquots of mRNA will be re-analyzed for IL-2 and IL-12.

(ii) PBMC from the two uninfected control monkeys showed high constitutive levels of IL-1b, as did PBMC from the four SIV-infected monkeys.

(iii) There appeared to be low but significant levels of IL-4, IL-6, IL-10, IFN-g, and TNF-b induction in the PHA-stimulated cultures from the two uninfected control monkeys.

**Conclusions and Future Plans:**

Infection and Viral Load analyses, Molecular Virology.

Necropsy specimens from the four macaques infected with SIVmac251 (see above) will be analyzed to determine cell and tissue distribution of virus and viral gene expression

(at UC Davis) and cytokine expression in various lymphoid organs (in collaboration with Dr. A. Ansari at Emory University).

Observation: We have made substantial progress on development of SIV/HIV-1 (SHIV) recombinant or chimeric viruses constructed by substituting the env-gp160 gene of SIVmac239 with env-gp160 genes (including tat, rev and vpu) of four unique HIV-1 isolates differing in several phenotypic properties (SF2, SF13, SF33, and SF162). $SHIV_{SF33}$, T-cell-line tropic and syncytium-inducing virus (SI phenotype), establishes a persistent infection in juvenile rhesus macaques. Importantly, one of four macaques persistently infected with $SHIV_{SF33}$ is displaying signs of SAIDS at 90 weeks post-infection (i.e., persistent generalized lymphadenopathy, wieght loss, neutropenia, lymphopenia, decline of CD4/CD8 cell ration from 0.8 to 0.35). Additionally, virus load is showing a dramatic increase at 90 weeks. Emerging opportunity: Transmission studies of $SHIV_{SF33}$ from this macaque to naieve macaques are planned. If such studies reveal pathogenesis, then we will emphasize construction of plasmid expression vectors containing HIV-$1_{SF33}$ env-gp160, with and without genes for cytokines. Should $SHIV_{SF33}$ prove to be reproducibly pathogenic in studies to be performed over the next six months, then the genetic immunization experiments will shift from SIVmac239 cloned DNA to HIV-$1_{SF33}$ cloned DNA. (21)

3.3. Observation: We have also discovered that a replication-competent SIV vector expressing the interleukin-2 gene (IL-2) causes a severe and fatal acute enteritis in newborn macaques. Analysis of necropsy materials reveals very high levels of virus in many tissues and very high numbers of both T-lymphocytes and monocytes in the GI tract and in lymph nodes. Taken together, these findings suggest that the IL-2 gene in the context of replicating virus has a dramatic physiological effect on the immune system. This notion supports the idea that IL-2, an perhaps other cytokines, co-expressed with SIV antigens will likely have a significant effect in vivo on anti-viral immune responses. Emerging opportunity: Continuing studies now involve analysis of the SIV-IL-2 vector in adult macaques which appear to show no disease signs but support moderate levels of vector virus; such studies are focused on measuring anti-viral immune responses, including levels of anti-viral antibodies, T-cell proliferation, and cytotoxic T-cell activity. Additionally, we will construct and test replication-competent vectors (expressing IL-12, IL-10, GM-CSF, etc.) in newborn and adult macaques. Such studies may reveal novel insights on cytokine function(s) and will provide data for comparison with macaques genetically immunized with plasmids co-expressing viral antigens and cytokine(s). (22).

Analyses of Humoral Immune Responses:

Since ELISA antigen was whole virus, the immune response measured may have mainly been directed against the envelope glycoprotein. Therefore, we plan to test the antibody reactivity against disrupted virus in order to include testing for antigens encoded by the gag and pol genes. This will be particularly important in the case of animal 26161 and 26470 in which no reactivity against the whole virus ELISA was observed. We are in the process of running Western blots to test the reactivity pattern of all the animals against all the proteins found in the virus particle. These data will be correlated with the ELISA results. We plan to do T cell proliferation assays against synthetic peptides representing major epitopes of each one of the major proteins found in the virus.

For the animals to be vaccinated with the DNA construct, we plan to run T cell proliferation using synthetic peptides representing defined epitopes on all the major proteins

coded by the DNA construct. For the control antigens in the T cell proliferation assay, we also plan to use irrelevant synthetic peptides.

Analyses of Cellular Immune Responses:

CTL responses to SIV were poorly detected in this experiment. An assay problem contributed to this. But it is possible that the intensive immunizations with tetanus and KLH (i.e antigenic stimulation) that were given shortly before SIV innoculation caused changes in lymphocyte priming and re-circulation, and thus altered the course of SIV infection. Problems with the assay of IL-2 and IL-12 cykine message levels from parallel PBMC cultures do not allow a correlation with these cytokines involved in the differentiation of CTL responses. In future experiments, to avoid the effects of imune activation on SIV challenge infection, immunizations of test antigens will be given over a longer time interval before SIV challenge.

Analysis of Cytokine Profiles:

The PBMC of the SIV-infected monkeys showed basically two major cytokine patterns. For one set of cytokines (IL-4, IFN-g), there were either low levels (IL-4) or moderate levels (IFN-g) of cytokine mRNA constitutively expressed, followed by a marked increase in IFN-g mRNA expression subsequent to PHA stimulation. For the other set of cytokines (IL-6, IL-10, and TNF-a), there were moderate levels of basal mRNA for each of these cytokines and marked increases for each following PHA stimulation. Of importance was the observation of a dramatic increase in the constitutive levels of IL-6 and TNF-a and, to a lower extent, IL-10 in the last sample that was analyzed from each of these four infected monkeys. Following stimulation with PHA, there was either no change, a slight increase, or a marked increase (seen in most samples) in the relative levels of mRNA for each of these cytokines.

Preparation of recombinant nonhuman primate cytokines: One of the objectives of Dr. Ansari's lab at Emory is to develop assays and reagents for quantitating nonhuman primate cytokines and growth factors and to prepare recombinant nonhuman cytokine gene constructs and soluble recombinant nonhuman primate cytokine proteins.

Dr. Ansari's laboratory has successfully prepared primer pairs and probes for the detection and quantitation of nonhuman primate IL-1a; IL-1b; IL-2, -3, -4, -6, -8, -10, -12a, -12b, -13, -14, -15; TNF-a; TNF-b; TGF-b; IFN-a; IFN-g; GM-CSF; Flt-3; Epo; TPO; C-CSF; Fas; and, for purposes of control, GAPDH. In addition, he has also prepared assays for the detection of IL-1, -2, -4, -6, -10, -12; TNF; and IFN-g proteins, and has prepared (using the CHO cell line and the pED4 vector) the following nonhuman recombinant cytokine proteins: IFN-g, IL-4, and IL-12. The data for IFN-g and IL-4 are described in {2418}, and the data on IL-12 are shown in Fig. 3a-g (attached). These show comparisons of recombinant rhesus IL-12 against recombinant human IL-12 (Fig. 3a); neutralization of rHu and rRh IL-12 by goat anti-human IL-12 (Fig. 3b), mAb 17F7 (Fig. 3c), mAb2-4A1 (Fig. 3D), and mAb 20C2 (Fig. 3e); and EIA for human and monkey IL-12, using mAb 4A1 (Fig. 3f) and mAb 20C2 (Fig. 3g). These data were derived in collaboration with Dr. Maurice Gately, Hoffman La Roche, Nutley, NJ.

Plans for year 2 of the project year: The consensus of the investigators of this program project is to focus attention during year 2 on optimizing dosage and route of administration of the protype DNA vaccine. The rationale for this strategy was based on the findings that there really has been no systematic study published, to date, that has carefully analyzed the

amount of DNA and the route of immunization that are required to induce and sustain an optimal humoral and/or cellular antigen-specific response in nonhuman primates. The role the investigators at Emory will be to:

(i) Assess quantitative levels of cytokines and cytokine mRNA in the PBMC of the monkeys immunized with the varying doses of the DNA vaccine administered by the various routes, as outlined in the protocol submitted by the P.I. Once again, control PBMC samples and PBMC samples stimulated *in vitro* for 24 hrs with PHA-P (2 mg/ml) or specific antigen will be pelleted and then shipped on dry ice from U.C. Davis to Emory. We will quantitate the relative levels of mRNA coding for rhesus IL-1a/b; IL-2, -4, -6, -10, -12; TNF-a; TNF-b; IFN-g; and, for purposes of control, GAPDH. Attempts will be made to correlate cytokine patterns with the quality and quantity of immune response of the vaccinated monkeys. It is thus possible that a DNA vaccine given sub-Q or I.M. vs intradermally may induce distinct cytokine profiles. Samples to be analyzed will include pre-immunization; 1 week post and 2 weeks post and 1 month, 3 months, 6 months, and 12 months post DNA vaccine immunization. There will be seven bleeds, and each bleed will constitute three sub-samples: control (*ex vivo*, PHA-P, and specific antigen). Thus, a total of 21 samples per monkey will be the subject of cytokine mRNa quantitation.

(ii) Continue to prepare IL-2, IFN-g, and IL-12 containing constructs of SIV: One of us (Dr. Paul Luciw) has already prepared a human rIL-2/SIV and a human rIFN-g/SIV and SHIV (HIV-1 env) constructs as alternate vaccine immunogens. We will provide the rhesus IL-2, IFN-g and IL-12 constructs, which will then be utilized to prepare rhesus IL-2, IFN-g, and IL-12 SIV and SHIV constructs. Once these are shown to be replication competent, they will then be utilized as vaccine candidates in rhesus macaques, utilizing the optimum schema as described above under 3(i).

**2) Develop novel expression vectors intended to improve levels of expression of SIV proteins in mice and primates.**

Vector Design.

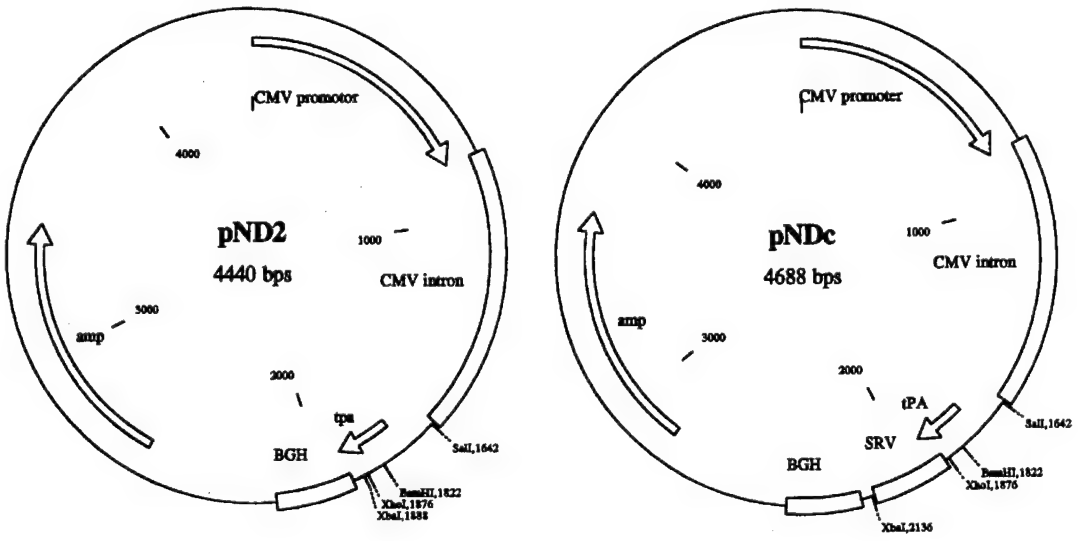
We have constructed two expression vectors to be used in the vaccination experiments, pND2 and pNDc. Maps of these two vectors are shown in Figure 2:1. They differ only in that pNDc contains a sequence named the constitutive transport element (CTE) from the virus SRV1 which allows expression of HIV (and SIV) late proteins in the absence of the HIV *rev* control protein ((23) and see below). Both vectors contain the following functional elements in common.

A. Promoter: The human cytomegalovirus IE1 promoter-intron was used as a promoter element because it has been shown to result in expression of large amounts of gp120 in tissue culture cells (24). It also gives the highest expression of 18 promoters tested after plasmid DNA injection into muscle ((25) and G. Rhodes, unpublished).

B. Terminator: The bovine growth hormone transcriptional terminator and polyadenylation region was chosen because it gives a 3-4 fold enhancement over a similar region derived from SV40 virus (26).

C. Heterologous signal sequence: Substitution of a heterologous signal sequence for the endogenous viral sequence at the beginning of HIV-1 gp120 results in a large increase in expression levels (24). We have included the signal sequence from human tissue plasminogen activator protein (TPA) in our vectors.

**Figure 2:1. Expression vectors pND2, pNDC.**



HIV gene expression is strictly controlled by several mechanisms. In particular, expression of HIV (and SIV) late genes requires the *rev* viral gene product. Expression of late genes is virtually nil in the absence of the *rev* protein because the viral mRNA is confined to the nucleus and is not available for translation in the cytoplasm (23, 27). Although expression of the gp120 portion of the *env* gene does not require *rev* function, expression of the full length gp160 region is completely *rev* dependent.

There are several strategies which have been used to express gp160 including the construction of expression vectors which contain both *env* and *rev* genes (28) or by mutation of the viral genes (27). However, the most general solution was first described by Hammarskjold and colleagues (23) who showed that the CTE cis-acting sequence from the class D group of retroviruses (they used the Mason-Pfizer virus) can be included in a heterologous message along with the HIV late genes to give *rev* independent expression. A similar functional element exists in the related virus SRV1 (29) and has been incorporated into the pNDc expression vector.

Expression vector construction.

A. **pUC19BGH** -- A 456 bp fragment containing the BGH terminator and part of a polylinker was amplified by PCR from a commercial plasmid (pcDNA3, Invitrogen) with a Nde I site inserted in the reverse primer. The product was digested with Nde I and Eco RI (an internal site) and the resulting 334 bp fragment was isolated and inserted into pUC19 which had been digested with these same two enzymes (Fig. 2:2).

B. **pND2** -- The CMV IE1 promoter-intron region and the TPA signal sequence was amplified by PCR from the pCMV-gp120 plasmid of Chapman et al (24) with Kpn I and Fsp I sites introduced into the primers. It was inserted into pUC19BGH which had been digested with Pvu II and Kpn I (Fig. 2:1).

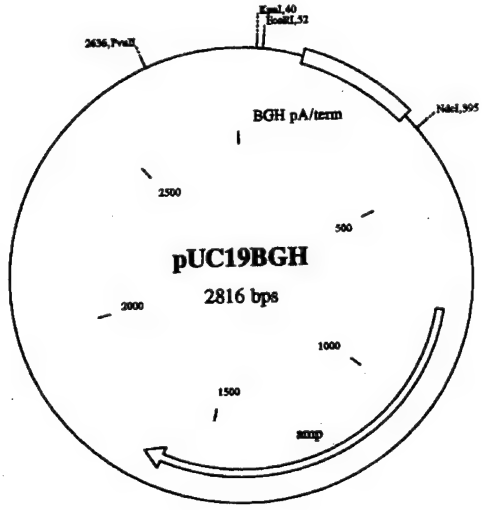
C. **pNDc** -- A 278 bp fragment from the SRV1 virus was amplified from the plasmid pSRV1 (29) with Xho I and Xba I sites incorporated into the end of the primers. This fragment was inserted into pND2 at these same sites (Fig. 2:1).

Insertion of reporter genes into expression vectors.

A. **pND2-lux and pNDc-lux** -- The coding region for firefly luciferase was inserted into both pND2 and pNDc after amplification from the commercial vector (pGL3I+, Promega) and incorporating Sal I and Bam HI sites.

B. **pND2-ßgal and pNDc-ßgal** -- The coding region for E. coli ß-galactosidase was amplified from a commercial plasmid (pCMVß-gal, Clonetech) incorporating Sal I and Bam HI sites.

**Figure 2:2. pUC19BGH**

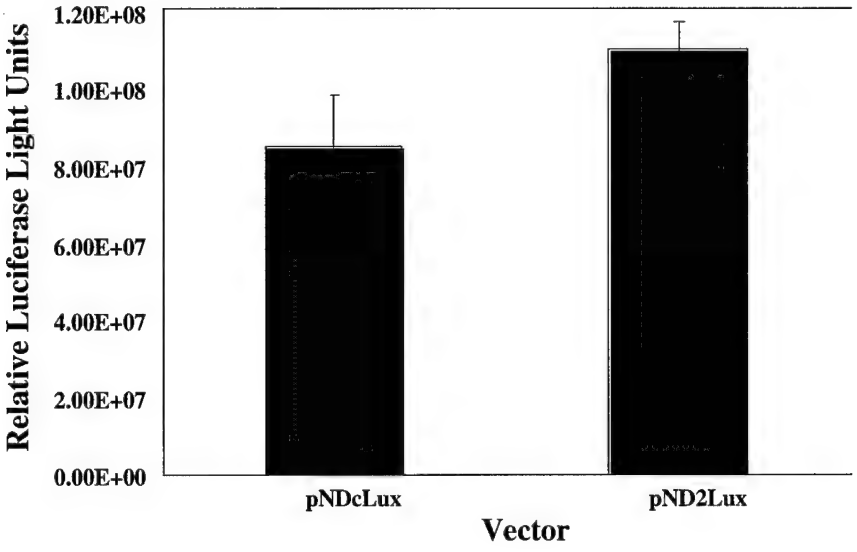



Testing of expression vectors

### A. Tissue culture transfection

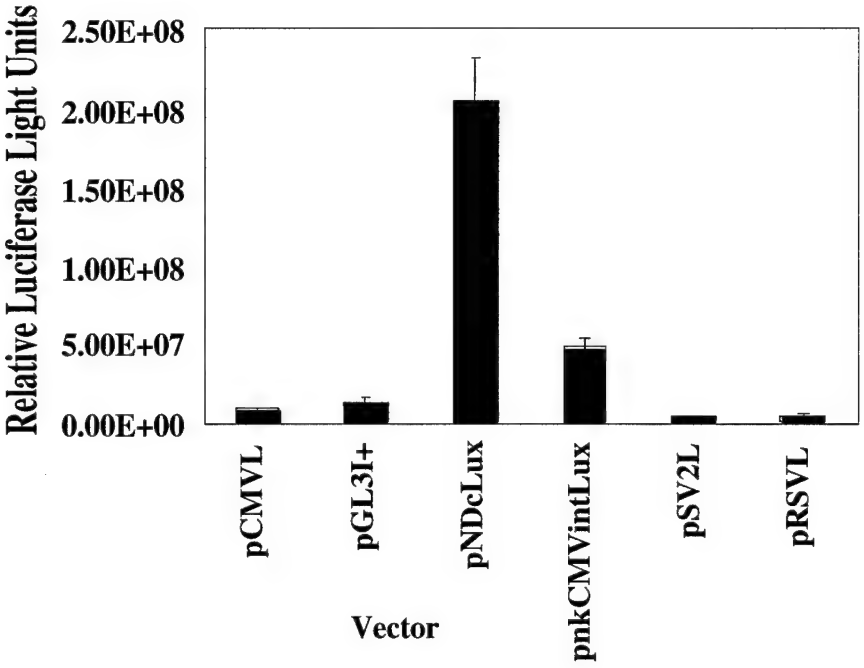
The plasmids pND2-lux and pNDc-lux were transfected into tissue culture cells using cationic lipids. One μg of plasmid DNA was added to an appropriate mass of a 50-50 mixture of the cationic lipid MLHME and the neutral lipid DOPE (30), yielding a 2:1 lipid:DNA charge ratio. The mixture was plated onto NIH 3T3 cells and luciferase levels were assayed after 48 hrs. The results of such an experiment are shown in Fig. 2:3 where we have compared the expression levels obtained with the two luciferase plasmids. Expression from both vectors is the same within experimental error indicating that both plasmids are functional.

**Figure 2:3. Comparison of activity of pND2-lux and pNDc-lux (NIH3T3 transfection).**




The data in Fig. 2:4 compare the expression levels obtained pNDc-lux to that to that obtained with some other luciferase expression vectors. The plasmid pCMVL contains the CMV IE promoter but no intron, pGL3I+ contains an SV40 late promoter, pSV2L contains the SV40 early promoter and pRSVL contains the Rous Sarcoma Virus LTR as promoter. The expression vector pnkCMVintLux has the CMV IE promoter and intron but also has the t intron from SV40 and a polyadenylation region from SV40. The same base expression vector has been used in many successful nucleic acid vaccination experiments (31). The figure shows that the vector developed for this grant functions as well or better than other standard expression vectors.

**Figure 2:4. Comparison of activity of pNDc-lux and previous expression vectors.**

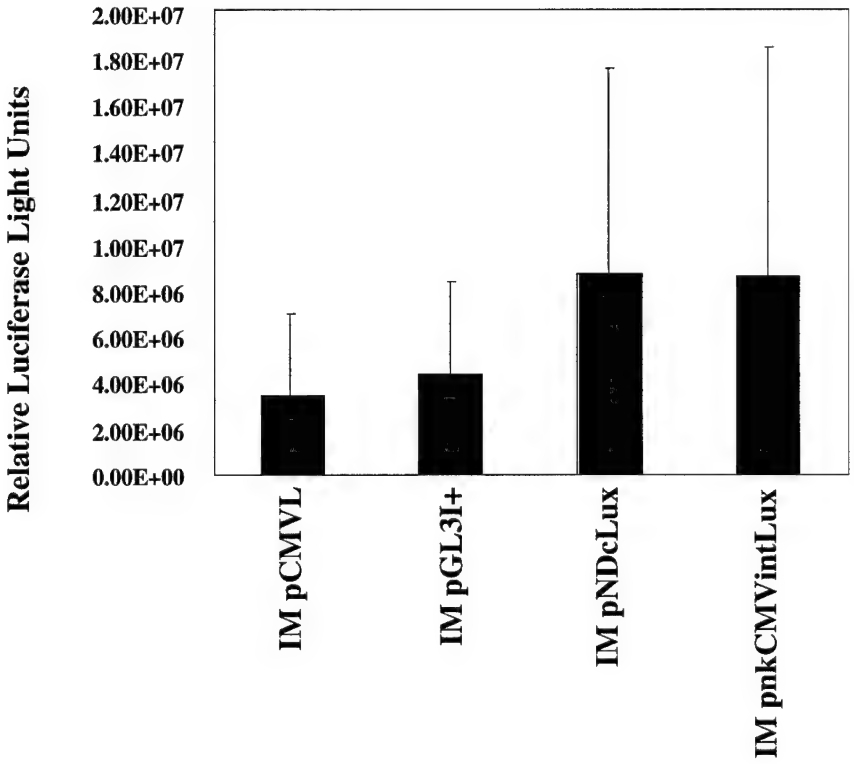



Similar transfection experiments were performed with the ß-galactosidase plasmids except that the cells were fixed and stained for enzymatic activity. Transfection with both vectors produced cells with ß-galactosidase activity indicating that both pND2 and pNDc were active (data not shown).

**Testing for *in vivo* expression**

Both luciferase plasmids were tested for expression after injection into mouse muscles. Plasmid DNA was resuspended in normal saline and 100 mg of DNA was injected into each quadracepts muscle. Three animals were injected with each plasmid. After 48 hours, muscles were removed and assayed for luciferase expression. The data in Fig. 6 compares the two luciferase plasmids and shows that both plasmids are about equally active *in vivo*. The data in Fig. 2:5 compares the pNDc-lux to other expression vectors in this assay. The new vector is as good or better than the other vectors tested.

**Figure 2:5. In vivo comparison of expression vectors.**




Currently in Progress

For reasons outlined in our research plan for next year, we have decided to study the immune response to several plasmids expressing portions of the SIV *env* gene. We have designed PCR primers to construct the following plasmids using the Bam HI and Eco RI sites in the pND2 and pNDc vectors.

1. The complete gp130 sequence.

2. The complete gp160 sequence.

3. The gp120 sequences plus additional sequences from the 5' end of gp41 which have been implicated in envelope dimerization.

4. The gp160 sequence with the carboxyterminal 42 amino acids removed. This region has been shown to be cytotoxic and to interact with calmodulin (32)

All three of these regions have been amplified and are currently being incorporated into into the expression vectors.

**General conclusions**

1. The plasmid expression vectors pND2 and pNDc actively express reporter genes both after tissue culture transfection and after direct intramuscular DNA injections in mice.

2. Expression levels observed with these plasmids is as good and in most cases higher than that seen with other expression vectors.

3. Expression of the SRV1 sequence does not have an adverse effect on expression of genes which are normally *rev* independent.

**Modified Research Plan, Year 2**

*Env* plasmids to be tested. We plan to test four envelope expression vectors that are illustrated below:

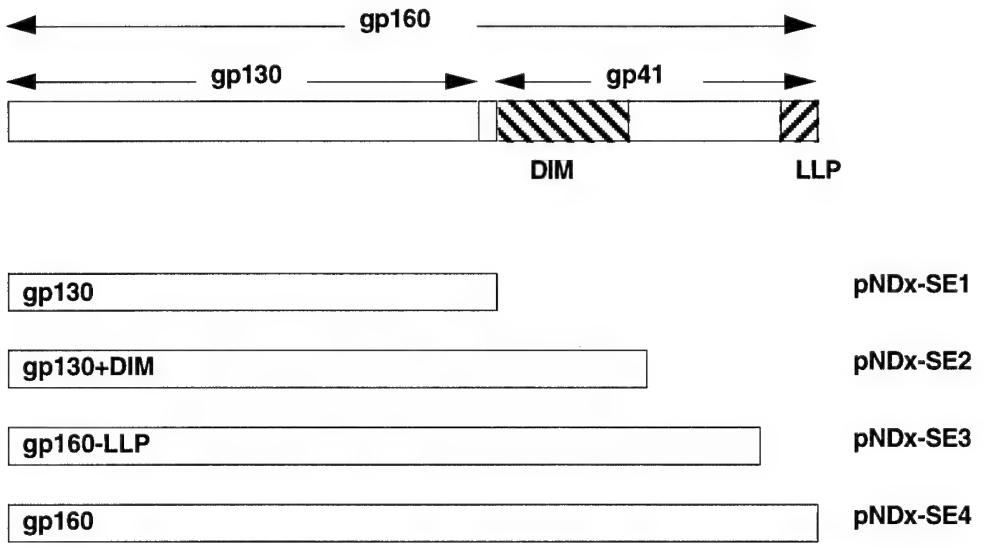



**Diagram of expression vectors containing portions of the *env* gene. DIM indicates the dimerization domain of gp41 and LLP indicates a region which is toxic to cells and which binds calmodulin.**

pNDx-SE1 contains just the gp130 region of the envelope gene.

pNDx-SE2 contains all of the gp130 region plus additional sequences in the N-terminal portion of gp41 which codes for a dimerization domain of the gp160 molecule. Our reasoning is that immunization with the gp130 protein has been unsuccessful in inducing

protective immunity. Although immunization with DNA containing gp130 may be more effective because of its ability to induce strong cellular immunity and a $Th_1$ response, it may also be that gp130 is in a conformation which is not conducive to the production of neutralizing antibodies. An extreme form of this hypothesis is that the gp130 is actually an immunological decoy as has been proposed by several authors. Inclusion of the dimerization domain will provide a soluble protein but in a dimer form and a confirmation which may differ substantially from gp130.

pNDx-SE3 contains all of gp160 except the C-terminal 42 amino acids. The deleted region is in the cytoplasmic tail of the protein and has been shown by Montelaro and coworkers to code for a peptide which is toxic to cells and which also binds to calmodulin. Our rational for testing this plasmid is that cells expressing this domain may be killed leading to transient antigen synthesis and a weak and/or temporary immune response. Cell death could be a direct result of peptide toxicity (unlikely) or because cells are activated as a consequence of the calmodulin interaction and are subsequently eliminated by the immune system.

pNDx-SE4 contains the full length gp160.

Primers have been designed for each of these plasmids and the regions have been amplified by PCR. We are currently inserting each fragment into both expression vectors, pND2 and pNDc.

Study of immune response in mice to a series of expression vectors containing portions of the *env* gene. We propose to test the four envelope constructs in mice and to measure both the peak antibody titers as well as the duration of the immune response. Selected mice will also be tested for CTL responses at both early and late times after vaccination. Groups of 6 mice will be injected with 50 mg of DNA in both quadracepts muscles. Groups of the same size will also be vaccinated intradermally with 20 mg of DNA. Serum samples will be collected at 3, 5, 8 and 12 weeks and antibody titers determined. CTL will be assayed in two mice from each group at 5 and 12 weeks. The expectation is that the gp130 plasmid will give a sustained and strong antibody response, the gp160 will give a weak and transient one.

Optimization of vaccination conditions in primates. We will use vectors expressing influenza NP, influenza HA and *E. coli* ß-galactosidase genes to optimize vaccination in primates. These antigens have been chosen because they are well studied in the mouse system in which they give lifetime immunity after a single injection, and we have developed ELISA assays for each in order to follow the immune response. The proposed experiments are designed to optimize the dose of DNA injected per animal, the injection volumes, and the route of injection (intradermal or intramuscular).

We will utilize 9 rhesus macaques for these studies, divided into three groups of three animals. The NP antigen will be used to test dose of DNA after intramuscular injection, ß-galactosidase to test the volume of injection and HA to test the amount of DNA to use in intradermal injections.

| Group | NP DNA | ß-gal DNA | HA DNA |
|---|---|---|---|
| A | mg DNA<br>ml volume | mg DNA<br>ml volume | mg DNA<br>ml volume |
| B | mg DNA<br>ml volume | mg DNA<br>ml volume | mg DNA<br>ml volume |
| C | mg DNA<br>ml volume | mg DNA<br>ml volume | mg DNA<br>ml volume |

Plasmid DNA will be purified by double CsCl banding, tested for endotoxin and dissolved in normal saline. Intramuscular injection will be into the biceps muscle group. Intradermal injection will be performed with a tuberculin syringe under the skin of the forearm. Serum samples will be collected at the day of injection and at two week intervals and assayed for antibodies to each antigen. The conditions giving the highest mean antibody titers will be used for subsequent SIV vaccination.

Primate immunization with SIV vectors and challenge. The four expression vectors will be injected into groups of animals using the optimum vaccination conditions as determined above. Animals will be followed at monthly intervals for at least 4 months to determine the stability and level of immunity. Serum samples will be collected at monthly intervals, antibody titers determined and CTL responses measured in selected animals. If the response is low or declines with time, the animals will be reinjected with plasmid. Other labs have shown that protection from challenge after infection with *nef* deletions is not observed for several months and we propose to wait this length of time before challenge. One month before challenge all animals will be reinjected with plasmid DNA and challenge and followup studies will be as described in the original grant proposal.

Revised research schedule.

Vector construction will be completed by the end of September 1995. Testing for expression after transfection will take place in October and the plasmid DNA to be used in these experiments will be purified at this time.

The mouse experiments will start in November 1995 and will be completed by March of 1996.

Vaccination optimization in primates will begin in January 1996 and will continue until April of 1996. Assay and data analysis will be completed by May 1996.

Immunization of primates with plasmid DNA will begin in May 1996. Assays of vaccinated animals will continue through September 1996. Reinjection of animals will be at the end of September and challenge at the end of October 1996.

Changes in the budget

Mouse charges. We will use 60 animals for 12 weeks. Costs are $ 5.00 per animals and $ 0.20 per day for housing. Total rodent costs are $ 908.00.

Primate rental charges. For optimization of DNA vaccination we will utilize 9 rhesus macaques for 12 to 14 weeks. Cost are $3.45 per day for each animal for a total cost of $ 3726.00. Additional costs for labor, supplies and overhead give a total cost of $7,087.44 (see attached memorandum from the California Primate Research Center).

References


1. L. Chakrabarti, M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, P. Sonigo, *Nature* **328**, 543-7 (1987).

2. G. Franchini, C. Gurgo, H. G. Guo, R. C. Gallo, E. Collalti, K. A. Fargnoli, L. F. Hall, F. Wong-Staal, M. Reitz Jr., *Nature* **328**, 539-43 (1987).

3. M. Fukasawa, T. Miura, A. Hasegawa, S. Morikawa, H. Tsujimoto, K. Miki, T. Kitamura, M. Hayami, *Nature* **333**, 457-61 (1988).

4. M. Kannagi, J. M. Yetz, N. L. Levtin, *Proc Natl Acad Sci USA* **82**, 7053-7 (1985).

5. N. L. Letvin, M. D. Daniel, P. K. Sehgal, R. C. Desrosiers, R. D. Hunt, L. M. Waldron, J. J. MacKey, D. K. Schmidt, L. V. Chalifoux, N. W. King, *Science* **230**, 71-3 (1985).

6. P. N. Fultz, H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, A. Srinivasan, *Proc Natl Acad Sci U S A* **83**, 5286-90 (1986).

7. M. Kannagi, M. Kiyotaki, R. C. Desrosiers, K. A. Reimann, N. W. King, L. M. Waldron, N. L. Letvin, *J Clin Invest* **78**, 1229-36 (1986).

8. M. Murphey-Corb, L. N. Martin, S. R. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, R. C. Montelaro, *Nature* **321**, 435-7 (1986).

9. G. B. Baskin, *Am J Pathol* **129**, 345-52 (1987).

10. M. D. Daniel, N. L. Letvin, P. K. Sehgal, G. Hunsmann, D. K. Schmidt, N. W. King, R. C. Desrosiers, *J Gen Virol* **68**, 3183-9 (1987).

11. G. B. Baskin, M. Murphey-Corb, E. A. Watson, L. N. Martin, *Vet Pathol* **25**, 456-67 (1988).

12. J. V. Torres, D. E. Anderson, A. Malley, B. Banapour, M. K. Axthelm, E. Benjamini, M. B. Gardner, *J Med Primatol* **22**, 129-37 (1993).

13. B. D. Forrest, *Infect Immun* **60**, 2023-9 (1992).

14. O. Nishio, J. Sumi, K. Sakae, Y. Ishihara, S. Isomura, S. Inouye, *Microbiol Immunol* **34**, 683-9 (1990).

15. T. Lehner, L. A. Bergmeier, C. Panagiotidi, L. Tao, R. Brookes, L. S. Klavinskis, P. Walker, J. Walker, R. G. Ward, L. Hussain, *Science* **258**, 1365-9 (1992).

16. M. D. Miller, S. Gould-Fogerite, L. Shen, R. M. Woods, S. Koenig, R. J. Mannino, N. L. Letvin, *J Exp Med* **176**, 1739-44 (1992).

17. P. A. Marx, R. W. Compans, A. Gettie, J. K. Staas, R. M. Gilley, M. J. Mulligan, G. V. Yamschikov, D. Chen, J. H. Eldridge, *Science* **260**, 1323-7 (1993).

18. C. J. Miller, D. W. Kang, M. Marthas, Z. Moldoveanu, H. Kiyono, P. Marx, J. H. Eldridge, J. Mestecky, J. R. McGhee, *Clin Exp Immunol* **88**, 520-6 (1992).

19. L. K. Borysiewicz, J. K. Hickling, S. Graham, J. Sinclair, M. P. Cranage, G. L. Smith, J. G. Sissons, *J Exp Med* **168**, 919-31 (1988).

20. M. D. Miller, C. I. Lord, V. Stallard, G. P. Mazzara, N. L. Letvin, *J Immunol* **144**, 122-8 (1990).

21. P. Luciw, et al. *Proc. Natl. Acad. Sci. USA* **92**, 7490-7494 (1995).

22. P. Luciw, et al. Pathogenesis by replication-competent simian immunodeficiency virus (SIV) vector expressing the interleukin-2 gene", 13th Annual Symposium on Nonhuman Primate Models for AIDS Monterey, CA, 1995),

23. M. Bray, S. Prasad, J. W. Dubay, E. Hunter, K. T. Jeang, D. Kekosh, M. L. Mannarskjold, *Proc Natl Acad Sci USA, in press* , (1994).

24. B. Chapman, R. Thayer, K. Vincent, N. Haigwood, *Nucleic Acids Research* **19**, 3979-86. (1991).

25. M. Manthorpe, F. Cornefert-Jensen, J. Hartikka, J. Felgner, A. Rundell, M. Margalith, V. Dwarki, *Hum Gene Ther* **4**, 419-31 (1993).

26. D. S. Pfarr, L. A. Rieser, R. P. Woychik, F. M. Rottman, R. M., M. E. Reff, *DNA* **5**, 115-122 (1986).

27. B. K. Felber, M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, G. N. Pavlakis, *Proc Natl Acad Sci U S A* **86**, 1495-9 (1989).

28. B. Wang, K. E. Ugen, V. Srikantan, M. G. Agadjanyan, K. Kang, A. L. Sato, Y. Refaeli, J. Boyer, W. V. Williams, D. B. Weiner, in *Vaccines 93* H. S. Ginsberg, F. Brown, R. M. Chanock, R. A. Lerner, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1993) pp. 143-150.

29. R. M. Thayer, r. M. D. Powe, M. L. Bryant, r. M. B. Gardner, P. J. Barr, P. A. Luciw, *Virology* **157**, 317-329. (1987).

30. R. P. Balasubramanian, M. J. Bennett, A. Aberle, J. Malone, M. Nantz, R. W. Malone, *Human Gene Therapy* , (In Press).

31. G. H. Rhodes, V. J. Dwarki, W. Chong, G. Ascardi, A. Jani, P. L. Felgner, in *Vaccines 93* H. S. Ginsberg, F. Brown, R. M. Chanock, R. A. Lerner, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1993) pp. 311-315.

32. M. A. Miller, T. A. Mietxner, M. W. Cloyd, W. G. Robey, R. C. Montelaro, *Aids Research and Human Retroviruses* **11**, 1057-66 (1993).

# Appendix 1:

# Results of cytokine analyses

**FIGURE 1 Control rhesus**

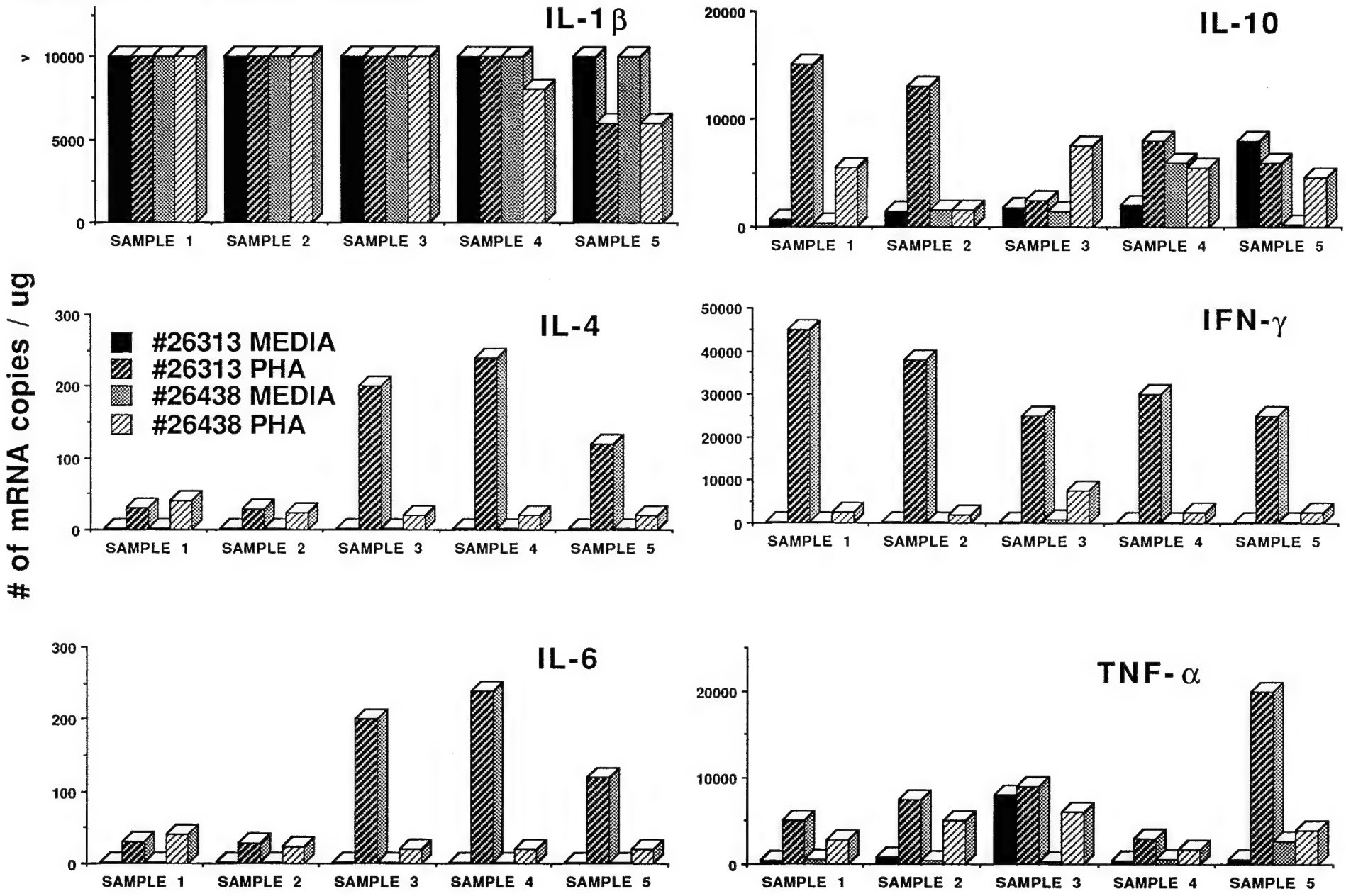


**FIGURE 2A SIVmac251 infected rhesus**

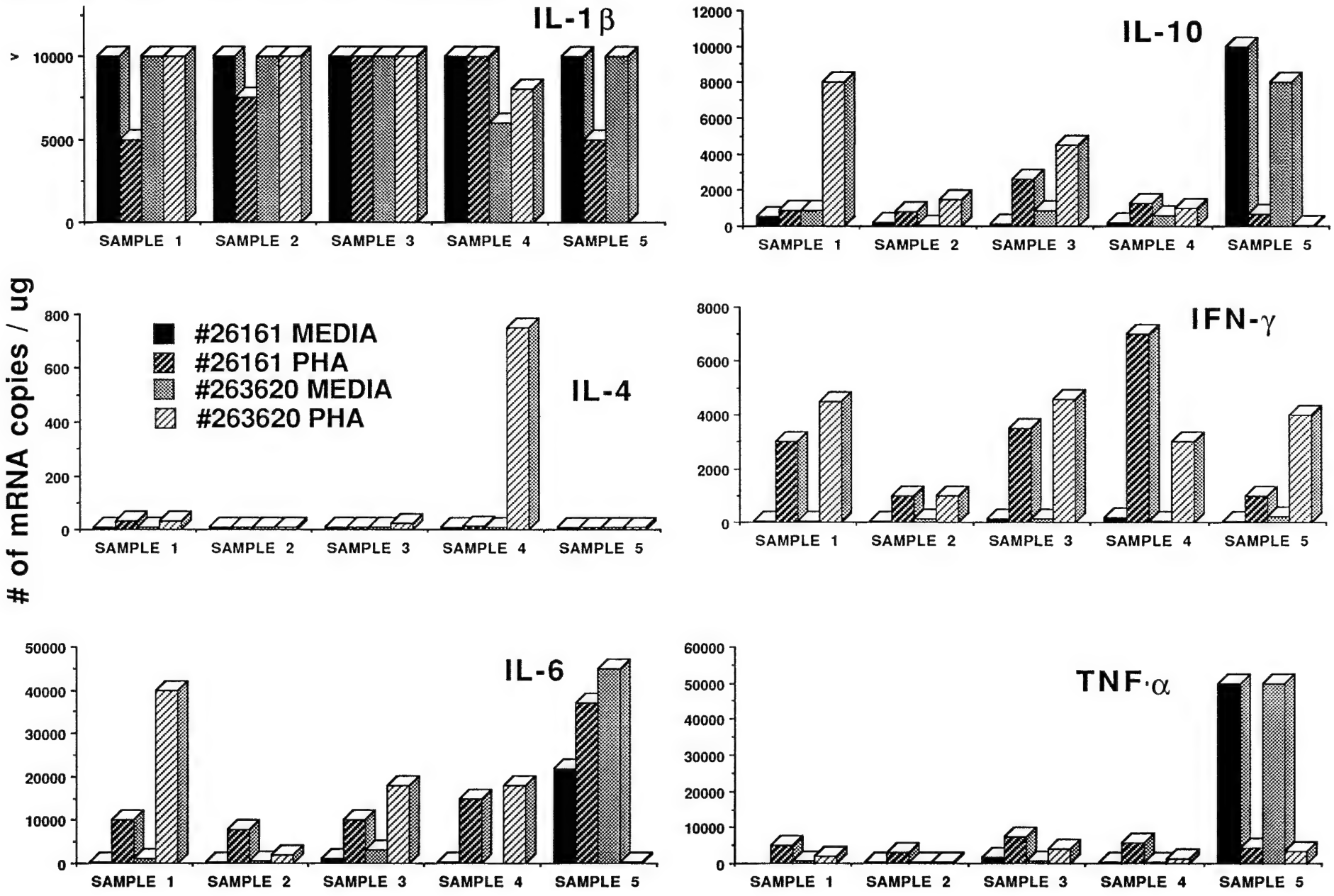


**FIGURE 2B SIVmac251 infected rhesus**

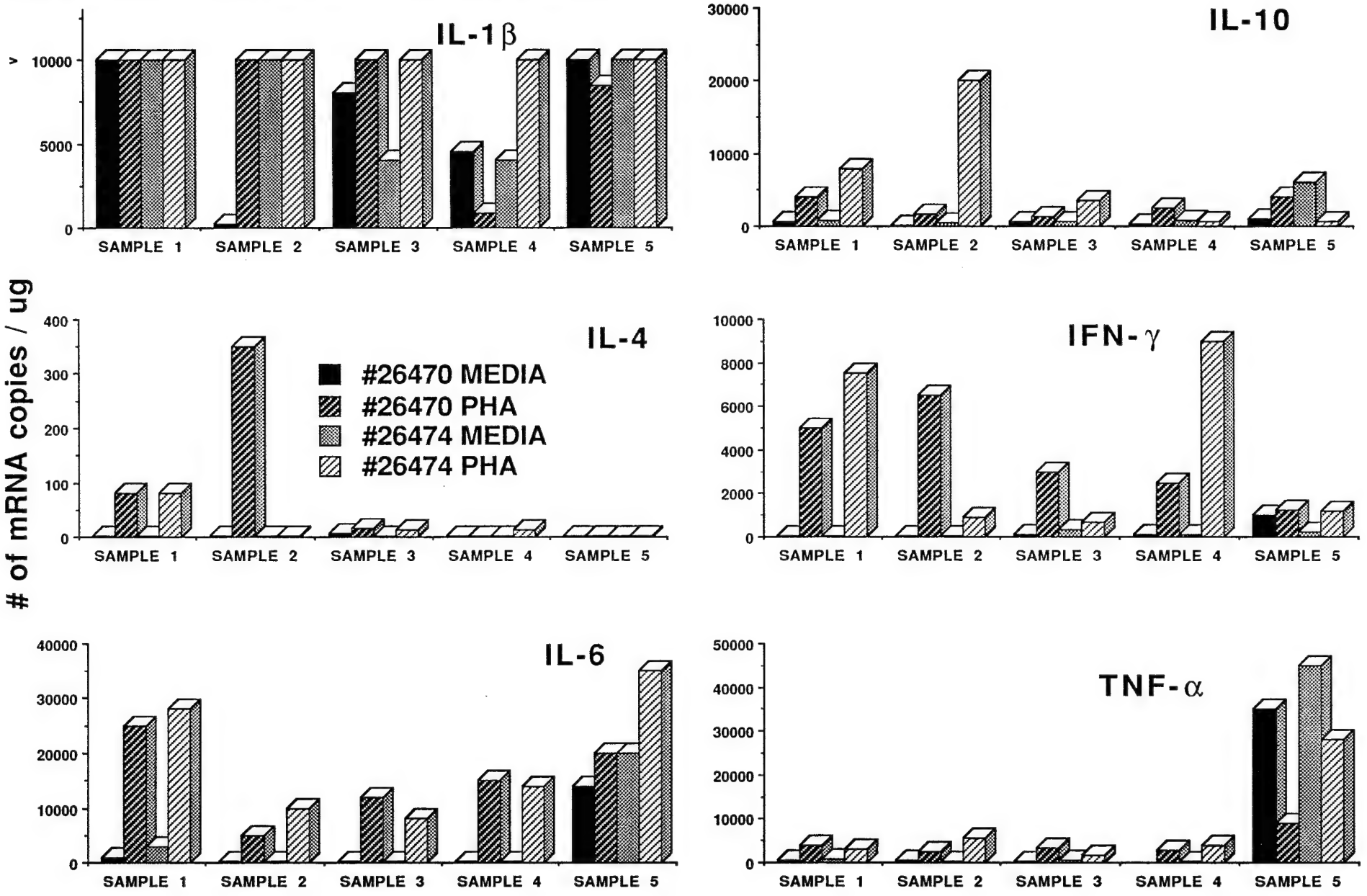


FIG. 3a

# MONKEY IL-12 VS HUMAN IL-12

## Human PHA Blasts

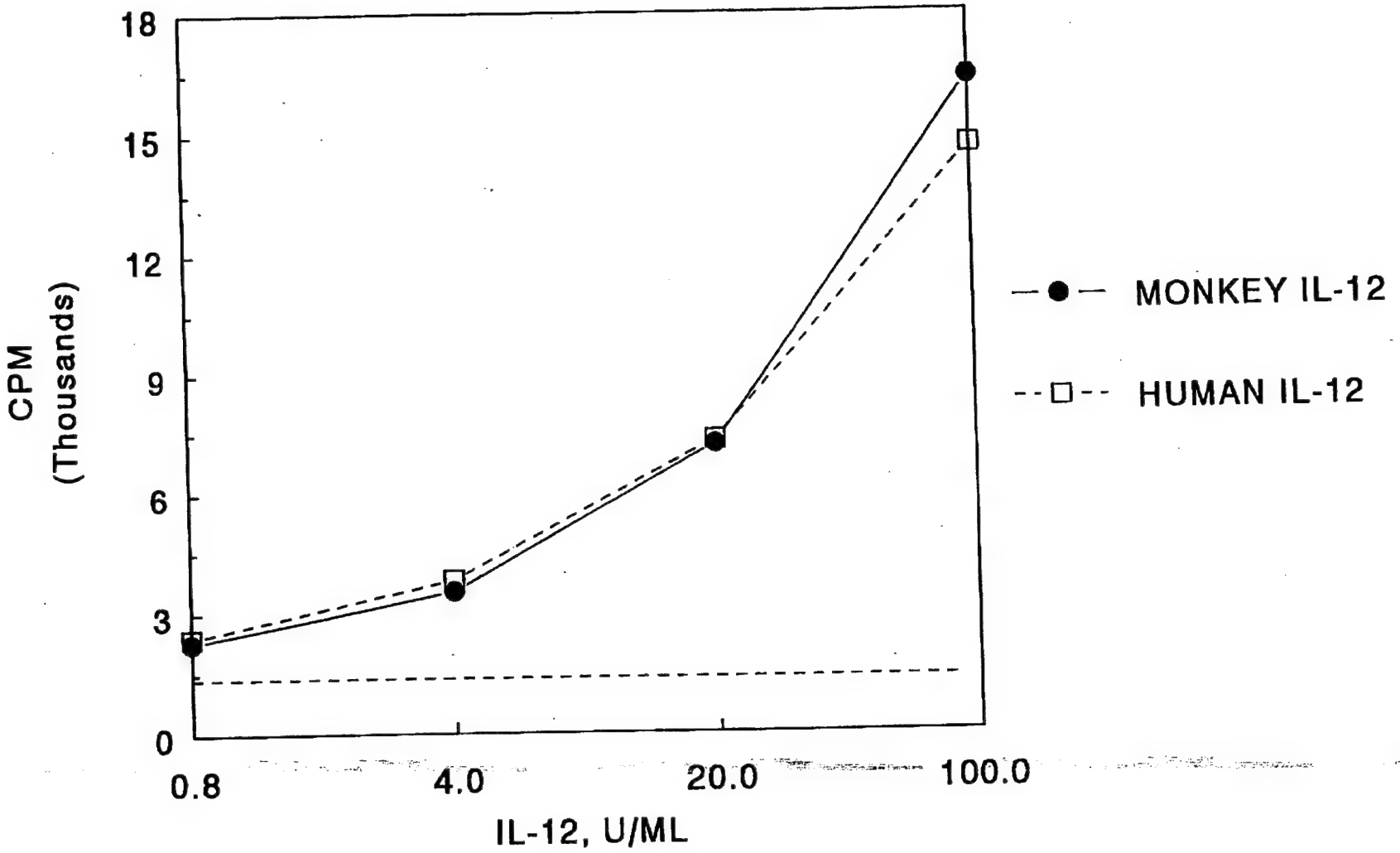


FIG. 3b

# Neutralization Of Human and Monkey IL-12 by Goat Anti-Human IL-12

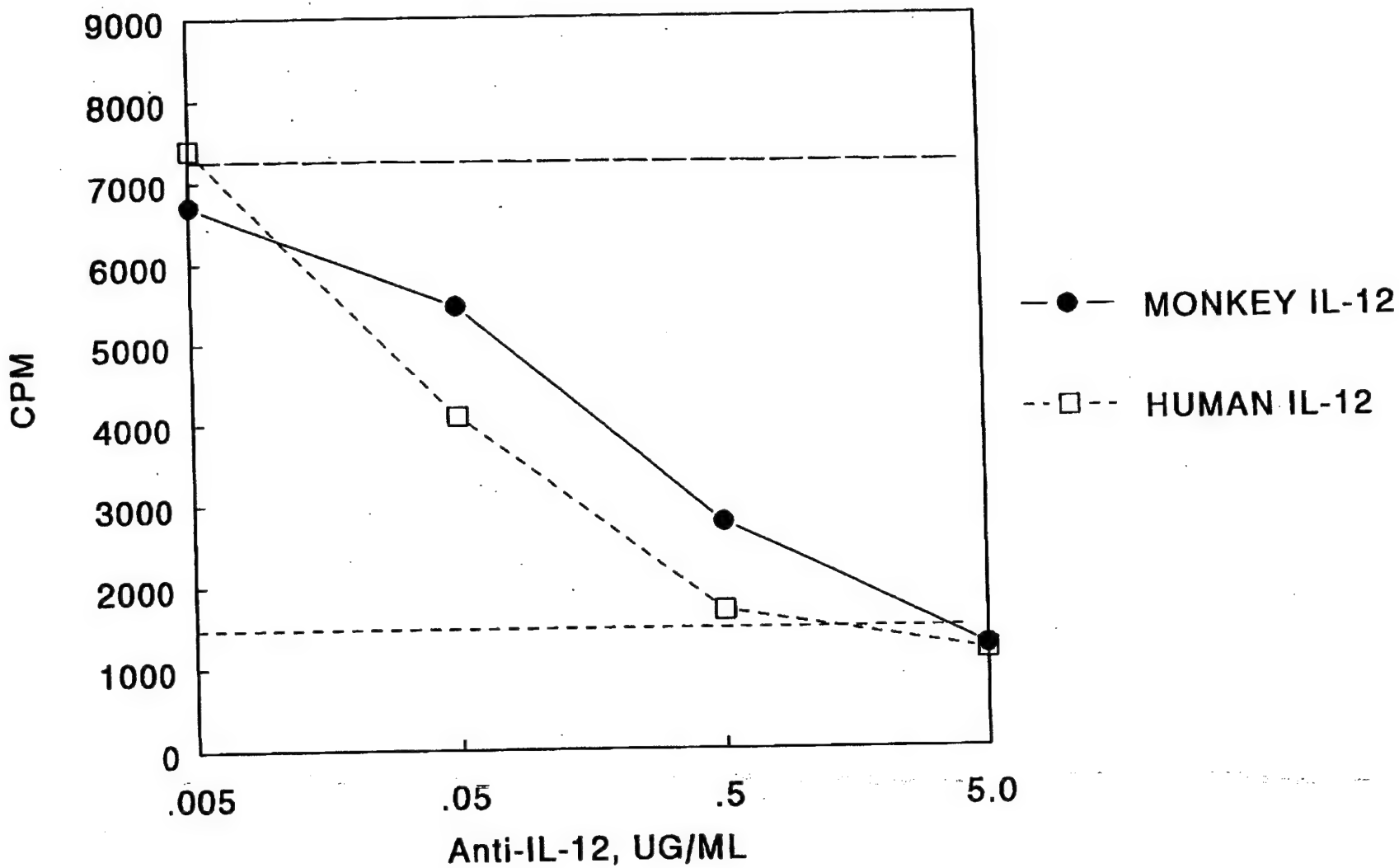


FIG. 3C

# Neutralization of Human and Monkey IL-12 by 17F7 Anti-Human IL-12

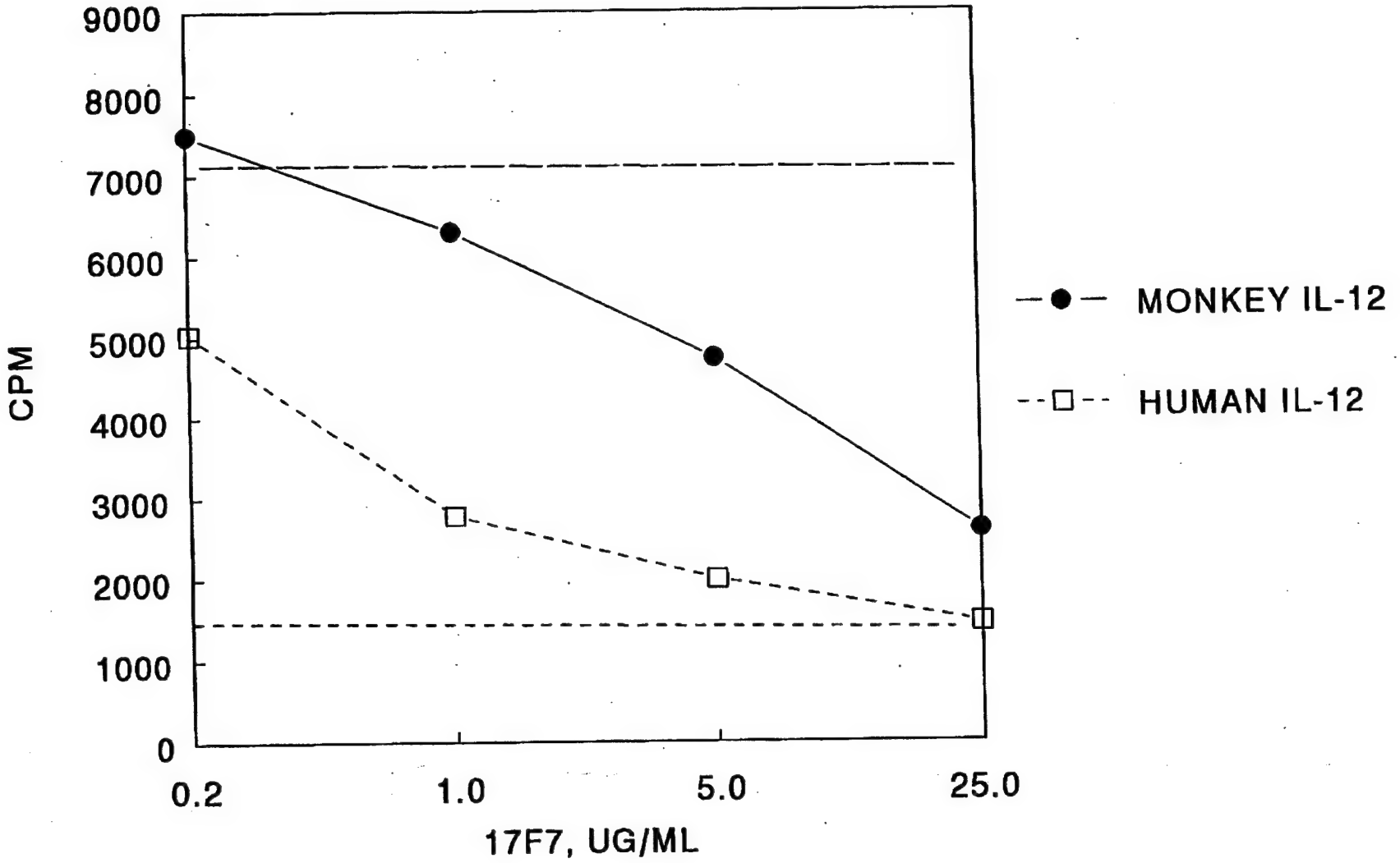


FIG. 3 d

# Neutralization of Human and Monkey IL-12 by 2-4A1 Anti-Human IL-12

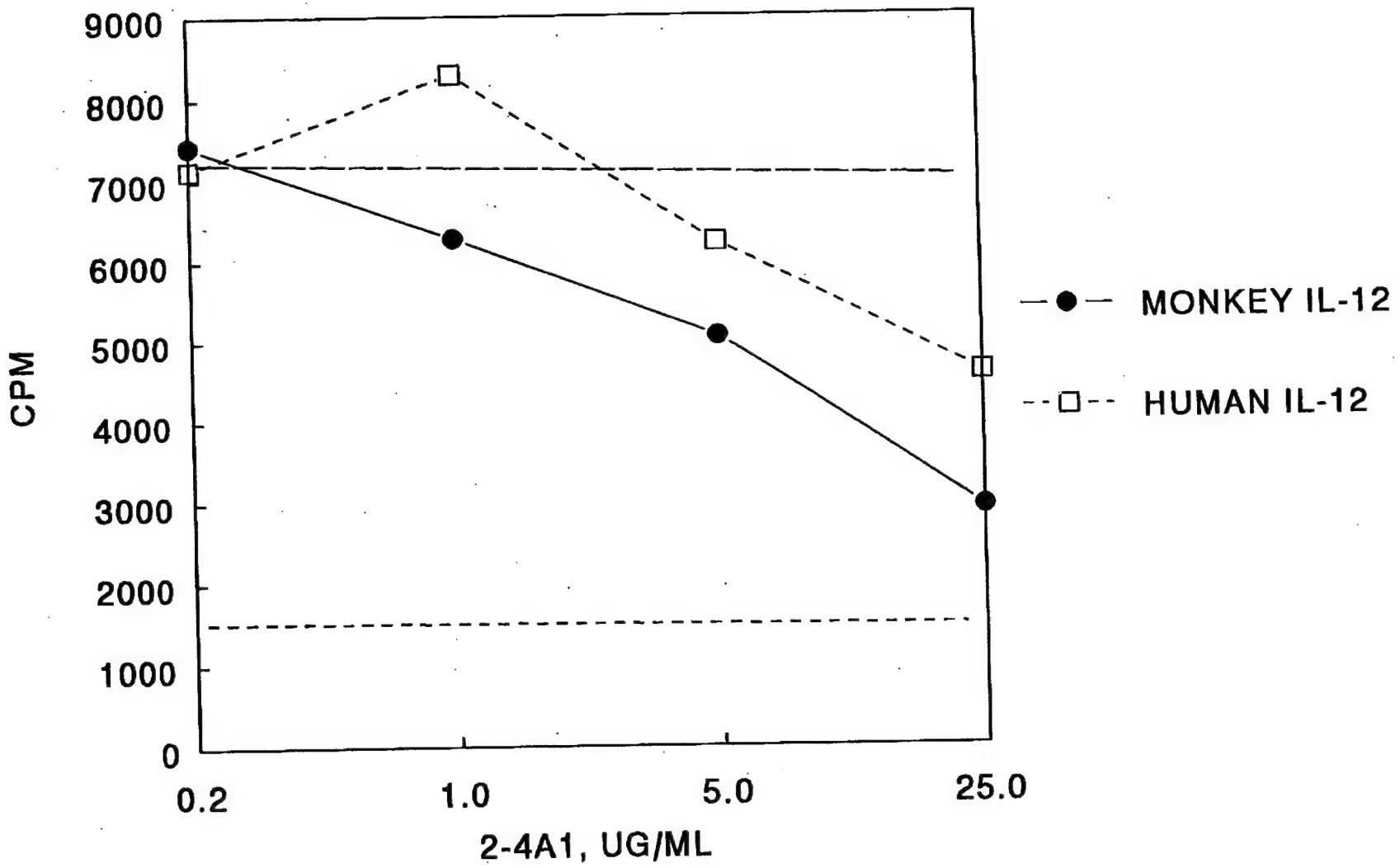


FIG. 3 e

# Neutralization Of Human and Monkey IL-12 by 20C2 Anti-Human IL-12

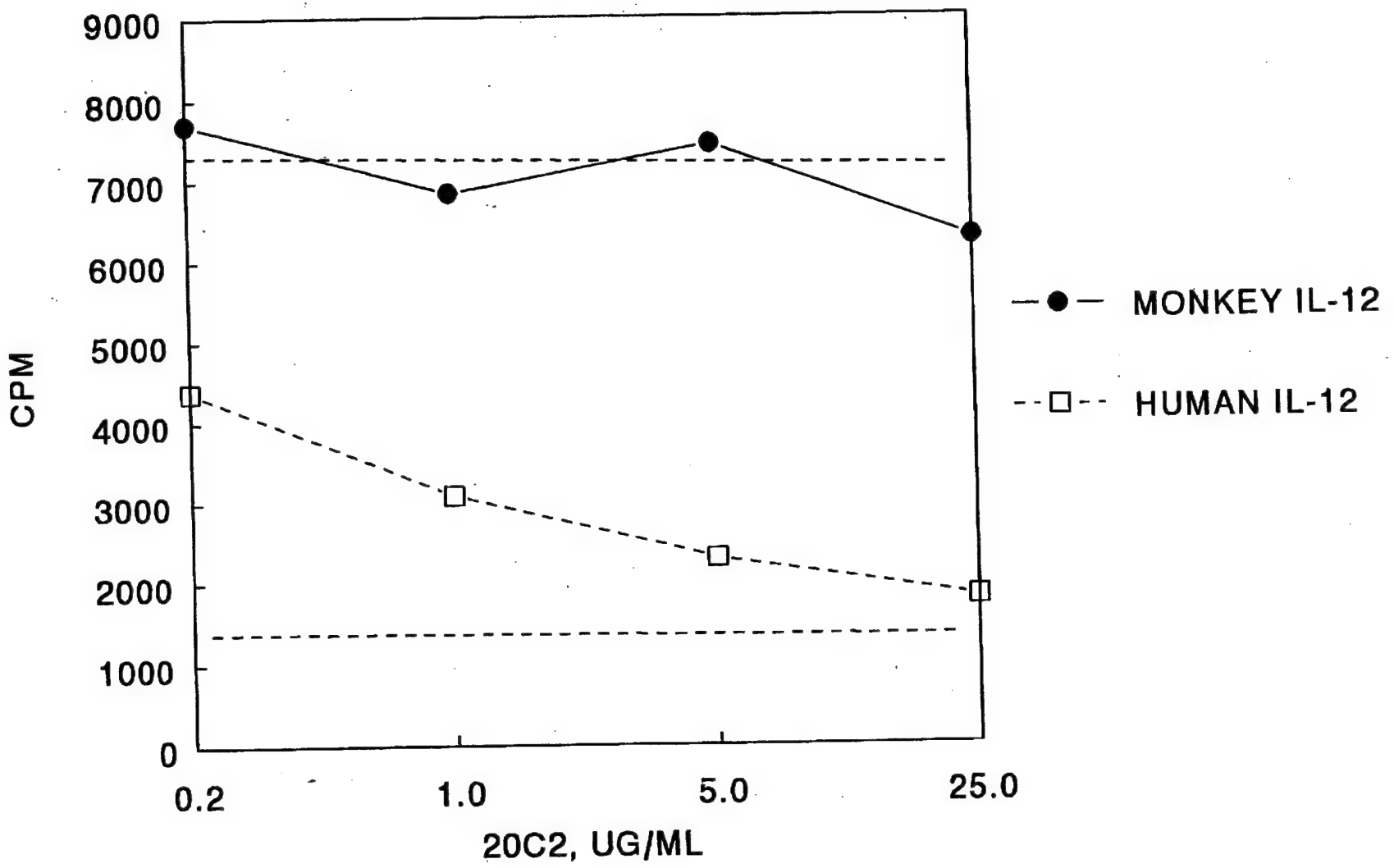


FIG. 3f

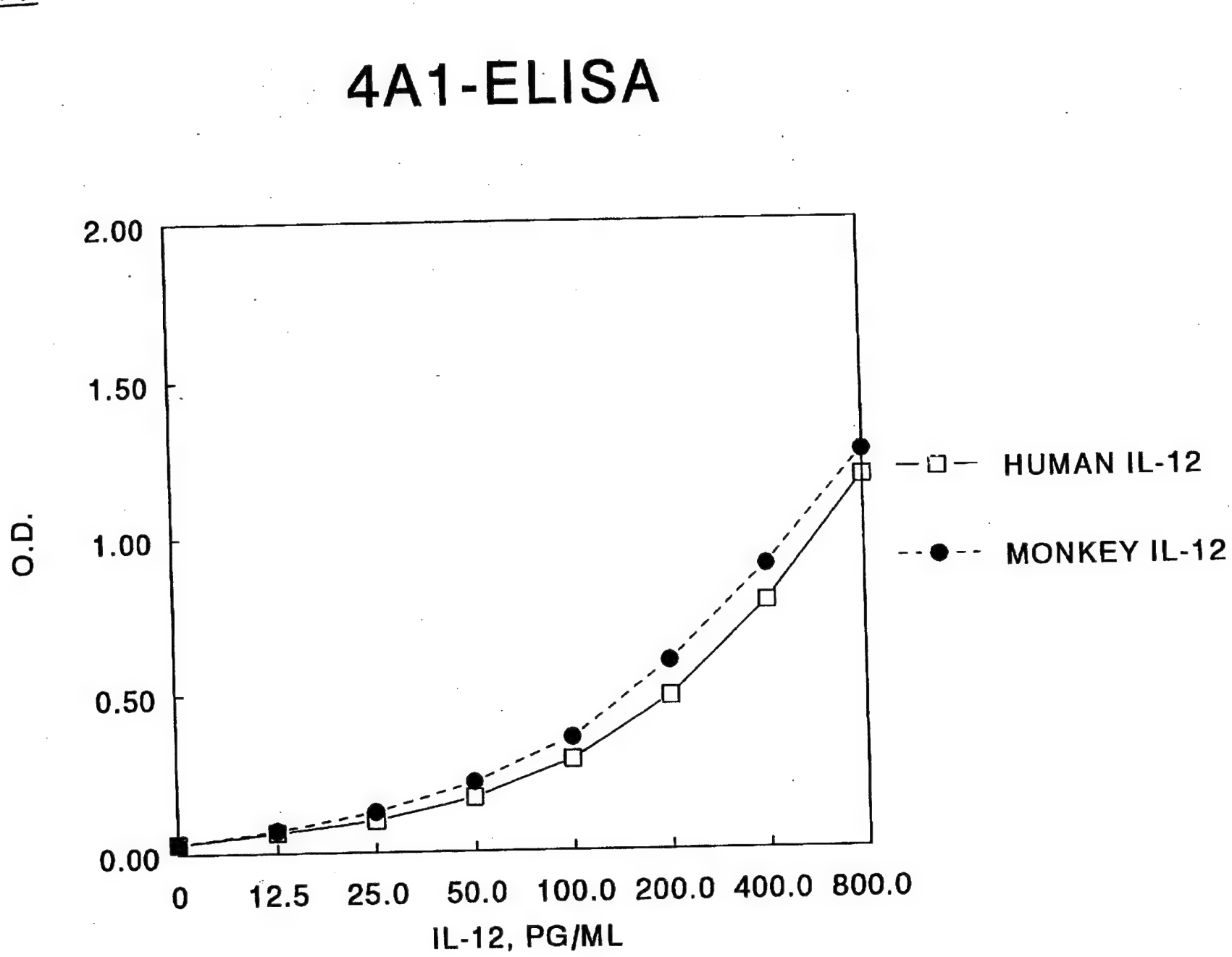
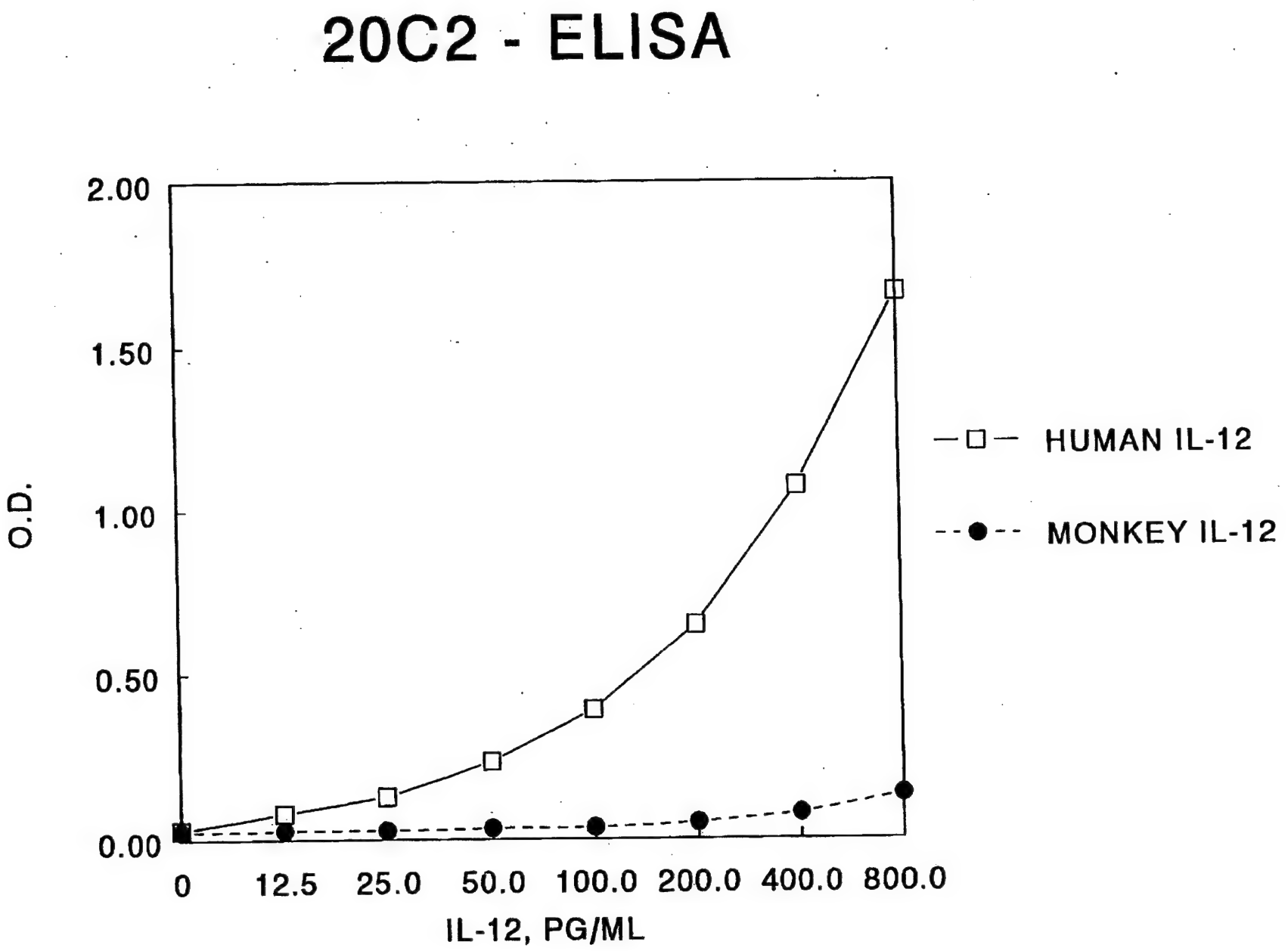

4A1-ELISA
2.00
1.50
1.00
0.50
0.00
O.D.
0
12.5
25.0
50.0
100.0
200.0
400.0
800.0
IL-12, PG/ML
HUMAN IL-12
MONKEY IL-12

FIG. 38


20C2 - ELISA
2.00
1.50
1.00
0.50
0.00
O.D.
0
12.5
25.0
50.0
100.0
200.0
400.0
800.0
IL-12, PG/ML
HUMAN IL-12
MONKEY IL-12

# Appendix 2:
# Results of cellular and humoral assays

| | | IgA | | IgG | |
|---|---|---|---|---|---|
| **Animal #** | **Antigen** | **1/24/95** | **4/18/95** | **1/24/95** | **4/18/95** |
| **26161** | TT | .385 | .131 | 2.134 | .186 |
| | KLH | 0 | .057 | 0 | 0 |
| | SIV | - | 0 | - | 0 |
| | | | | | |
| **26313** | TT | .566 | .302 | 2.083 | .257 |
| | KLH | 0 | .096 | 0 | .042 |
| | SIV | - | 0 | - | 0 |
| | | | | | |
| **26320** | TT | .660 | 0 | 2.118 | 0 |
| | KLH | .169 | 0 | 0 | 0 |
| | SIV | - | 0 | - | 0 |
| | | | | | |
| **26438** | TT | .881 | 0 | 1.952 | 0 |
| | KLH | .253 | 0 | 0 | 0 |
| | SIV | - | 0 | - | 0 |
| | | | | | |
| **26470** | TT | .740 | .264 | 1.912 | .659 |
| | KLH | 0 | 0 | .127 | .136 |
| | SIV | - | 0 | - | .281 |
| | | | | | |
| **26474** | TT | .702 | .019 | 1.587 | .050 |
| | KLH | 0 | .008 | 0 | .045 |
| | SIV | - | 0 | - | .043 |

## IgA and IgG SECRETION BY MACAQUE PBMC

All values are adjusted by subtracting values obtained with a control irrelevant peptide.
- Indicates not done.

# MMU 26161: Proliferation Summary

| **12/27** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **3.45** | **4.94** | **3.45** | 1.75 |
| tetanus toxoid | **2.06** | **2.88** | **2.28** | 1.03 |
| gp130 | 0.44 | 0.41 | 0.47 | 0.56 |

**12/27**: immunized with KLH and tetanus toxoid

| **1/10** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **5.05** | **4.99** | **2.83** | 1.88 |
| tetanus toxoid | 1.51 | **2.93** | **2.30** | 1.29 |
| gp130 | 1.67 | 1.88 | 1.21 | 1.92 |

**1/10**: boosted with KLH, immunized with flu vaccine

| **1/24** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.82 | 1.87 | **2.02** | 1.21 |
| tetanus toxoid | 1.84 | 1.13 | 1.58 | 1.36 |
| gp130 | 1.87 | 0.97 | 1.66 | 1.08 |

**1/24**: boosted with KLH and tetanus toxoid

| **2/7** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **2.01** | 1.79 | **2.27** | 1.39 |
| tetanus toxoid | 1.47 | **2.21** | **4.45** | **2.45** |
| gp130 | **2.20** | 1.67 | 1.27 | 0.94 |

**2/7**: inoculated with SIV

| **2/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.30 | **3.11** | 1.90 | 0.68 |
| tetanus toxoid | **2.59** | 1.30 | 1.18 | 0.54 |
| gp130 | 1.09 | 1.18 | 1.42 | 1.09 |

| | SI | SI | SI | SI |
|---|---|---|---|---|
| KLH | **2.19** | 1.25 | **21.02** | **2.77** |
| tetanus toxoid | 1.74 | 1.06 | 0.66 | 0.62 |
| gp130 | 1.27 | 0.72 | 0.82 | 0.89 |

| **4/18** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **9.06** | **3.97** | 1.74 | 1.08 |
| tetanus toxoid | 1.15 | **2.59** | 1.26 | 0.55 |
| gp130 | 0.80 | 0.66 | 1.06 | 0.75 |

Prepared by Dave Nabi 8/25/95

# MMU 26313 (negative control): Proliferation Summary

| **12/27** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.57 | 0.50 | 0.62 | 0.30 |
| tetanus toxoid | 0.20 | 0.41 | 0.46 | 0.24 |
| gp130 | 0.92 | 0.74 | 1.24 | 1.49 |

**12/27**: immunized with KLH and tetanus toxoid

| **1/10** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **3.26** | **3.59** | **2.68** | 1.52 |
| tetanus toxoid | **2.47** | 1.61 | 1.44 | 0.92 |
| gp130 | 0.99 | 0.70 | 1.13 | 1.00 |

**1/10**: boosted with KLH, immunized with flu vaccine

| **1/24** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **20.45** | **21.91** | **17.44** | **23.26** |
| tetanus toxoid | **2.37** | **3.53** | **4.60** | **3.68** |
| gp130 | **5.71** | **2.28** | **2.98** | 1.92 |

**1/24**: boosted with KLH and tetanus toxoid

| **2/7** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.97 | 1.35 | **2.18** | 0.96 |
| tetanus toxoid | 0.80 | 0.83 | **2.45** | 1.03 |
| gp130 | 1.80 | 0.79 | 1.14 | 0.79 |

| **2/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **4.28** | **7.17** | **6.76** | **2.12** |
| tetanus toxoid | **7.55** | **2.80** | 1.27 | **2.58** |
| gp130 | XXX | 0.79 | 0.87 | 1.17 |

| **3/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **7.92** | **7.00** | **4.49** | **3.70** |
| tetanus toxoid | **7.16** | **3.06** | **3.29** | **2.66** |
| gp130 | 0.23 | 0.66 | 0.46 | 0.83 |

| **4/18** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **3.41** | 1.91 | 1.09 | 0.51 |
| tetanus toxoid | 1.82 | 1.66 | 0.97 | 0.73 |
| gp130 | 0.71 | 0.56 | 1.21 | 0.63 |

Prepared by Dave Nabi 8/25/95

# MMU 26320: Proliferation Summary

| **12/27** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.22 | 0.39 | 0.64 | 0.45 |
| tetanus toxoid | **56.96** | 0.53 | 0.40 | 0.24 |
| gp130 | 0.18 | 0.21 | 0.34 | 0.31 |

**12/27**: immunized with KLH and tetanus toxoid

| **1/10** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **2.82** | 1.88 | 1.58 | 1.27 |
| tetanus toxoid | 1.84 | 0.73 | 1.34 | 0.96 |
| gp130 | 0.86 | 0.48 | 0.78 | 0.63 |

**1/10**: boosted with KLH, immunized with flu vaccine

| **1/24** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **4.02** | **3.85** | **3.86** | 1.44 |
| tetanus toxoid | **2.21** | 1.42 | 1.23 | 1.35 |
| gp130 | 1.84 | **3.24** | 1.56 | 1.30 |

**1/24**: boosted with KLH and tetanus toxoid

| **2/7** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.42 | 0.86 | 1.20 | 0.91 |
| tetanus toxoid | 0.75 | 0.92 | 1.00 | 0.96 |
| gp130 | 1.39 | 1.64 | 1.18 | 0.67 |

**2/7**: inoculated with SIV

| **2/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **8.99** | **12.92** | **2.77** | 0.99 |
| tetanus toxoid | 0.96 | 1.74 | **2.81** | 1.01 |
| gp130 | 0.65 | 1.25 | 0.79 | 0.94 |

| **3/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **6.05** | **6.34** | **3.80** | 0.81 |
| tetanus toxoid | 1.53 | 1.11 | 0.89 | 0.79 |
| gp130 | 0.61 | 1.07 | 0.78 | 0.50 |

| **4/18** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **2.75** | 1.28 | 0.93 | 0.57 |
| tetanus toxoid | **2.00** | 0.92 | 0.73 | 0.52 |
| gp130 | 0.95 | 0.74 | 0.99 | 0.58 |

Prepared by Dave Nabi 8/25/95

# MMU 26438 (negative control): Proliferation Summary

| **12/27** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.93 | 1.09 | 1.06 | 0.97 |
| tetanus toxoid | **38.34** | 0.76 | 0.31 | 0.39 |
| gp130 | 1.11 | 1.23 | 1.21 | 1.10 |

**12/27**: immunized with KLH and tetanus toxoid

| **1/10** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.65 | 0.62 | 0.42 | 0.27 |
| tetanus toxoid | 0.57 | 0.40 | 0.34 | 0.22 |
| gp130 | 0.47 | 0.48 | 0.34 | 0.25 |

**1/10**: boosted with KLH, immunized with flu vaccine

| **1/24** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **4.88** | **2.95** | **3.62** | **2.74** |
| tetanus toxoid | **2.65** | 2.35 | 1.57 | 1.08 |
| gp130 | **3.10** | **3.45** | **3.21** | 1.12 |

**1/24**: boosted with KLH and tetanus toxoid

| **2/7** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.03 | 0.96 | 1.83 | 1.03 |
| tetanus toxoid | 0.68 | 0.75 | **3.99** | 1.28 |
| gp130 | **4.62** | **2.55** | 1.61 | 0.63 |

| **2/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.82 | 1.84 | **2.95** | 0.54 |
| tetanus toxoid | **2.38** | 1.47 | **2.74** | 0.71 |
| gp130 | XXX | 1.15 | 1.38 | 0.69 |

| **3/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **2.63** | 1.01 | 1.74 | 1.20 |
| tetanus toxoid | 1.57 | 1.46 | 1.13 | 0.64 |
| gp130 | 0.16 | 0.62 | 0.40 | 0.44 |

| **4/18** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.48 | 0.90 | 1.09 | 1.12 |
| tetanus toxoid | 1.44 | 1.20 | 1.31 | 0.89 |
| gp130 | 0.39 | 0.46 | 1.04 | 1.16 |

Prepared by Dave Nabi 8/25/95

# MMU 26470: Proliferation Summary

| **12/27** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | 1.64 | 1.45 | 1.48 | 0.88 |
| tetanus toxoid | | 1.18 | 1.82 | 1.69 | 0.59 |
| gp130 | | 1.07 | 0.97 | 0.52 | 0.87 |

**12/27**: immunized with KLH and tetanus toxoid

| **1/10** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | 1.66 | 1.48 | 1.30 | 0.83 |
| tetanus toxoid | | 1.50 | 1.88 | 1.33 | 1.00 |
| gp130 | | 1.40 | **2.09** | 1.34 | **2.30** |

**1/10**: boosted with KLH, immunized with flu vaccine

| **1/24** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | **4.15** | **2.77** | 1.60 | **2.61** |
| tetanus toxoid | | 1.83 | 1.18 | 1.09 | 1.03 |
| gp130 | | **3.40** | 1.40 | 1.16 | 0.78 |

**1/24**: boosted with KLH and tetanus toxoid

| **2/7** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | 0.76 | 1.02 | 1.34 | 0.67 |
| tetanus toxoid | | 0.71 | 1.03 | 1.71 | **3.06** |
| gp130 | | 0.97 | 1.22 | 1.64 | 0.76 |

**2/7**: inoculated with SIV

| **2/21** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | 1.14 | **2.57** | 1.23 | 0.67 |
| tetanus toxoid | | 0.85 | 0.73 | 0.64 | 0.56 |
| gp130 | | 0.72 | 0.86 | 0.60 | 0.64 |

| **3/21** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | **13.99** | **10.83** | **2.63** | 0.58 |
| tetanus toxoid | | 1.02 | 1.03 | 0.85 | 0.49 |
| gp130 | | 0.58 | 0.50 | 1.02 | 0.72 |

| **4/18** | ug/mL: | 10.0<br>SI | 3.3<br>SI | 1.0<br>SI | 0.1<br>SI |
|---|---|---|---|---|---|
| KLH | | **6.61** | **3.01** | **2.76** | 1.91 |
| tetanus toxoid | | **3.36** | **3.01** | **2.46** | 1.93 |
| gp130 | | 1.19 | 0.76 | 1.11 | 0.64 |

Prepared by Dave Nabi 8/25/95

# MMU 26474: Proliferation Summary

| **12/27** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.09 | 1.40 | 1.11 | 0.55 |
| tetanus toxoid | 0.58 | 1.02 | 0.68 | 0.62 |
| gp130 | 0.74 | 0.86 | 0.41 | 0.30 |

**12/27**: immunized with KLH and tetanus toxoid

| **1/10** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.88 | 1.07 | 1.39 | 0.87 |
| tetanus toxoid | 0.88 | **2.50** | 1.02 | 0.77 |
| gp130 | 1.00 | 1.70 | 0.95 | 0.81 |

**1/10**: boosted with KLH, immunized with flu vaccine

| **1/24** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.43 | 1.06 | 1.20 | 0.74 |
| tetanus toxoid | 0.66 | 0.93 | 0.58 | 0.77 |
| gp130 | 1.78 | 1.33 | 1.06 | 1.02 |

**1/24**: boosted with KLH and tetanus toxoid

| **2/7** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 0.68 | 0.83 | 0.87 | 0.88 |
| tetanus toxoid | 0.82 | 0.88 | 0.72 | 0.90 |
| gp130 | 1.28 | **2.15** | 1.96 | 0.56 |

**2/7**: inoculated with SIV

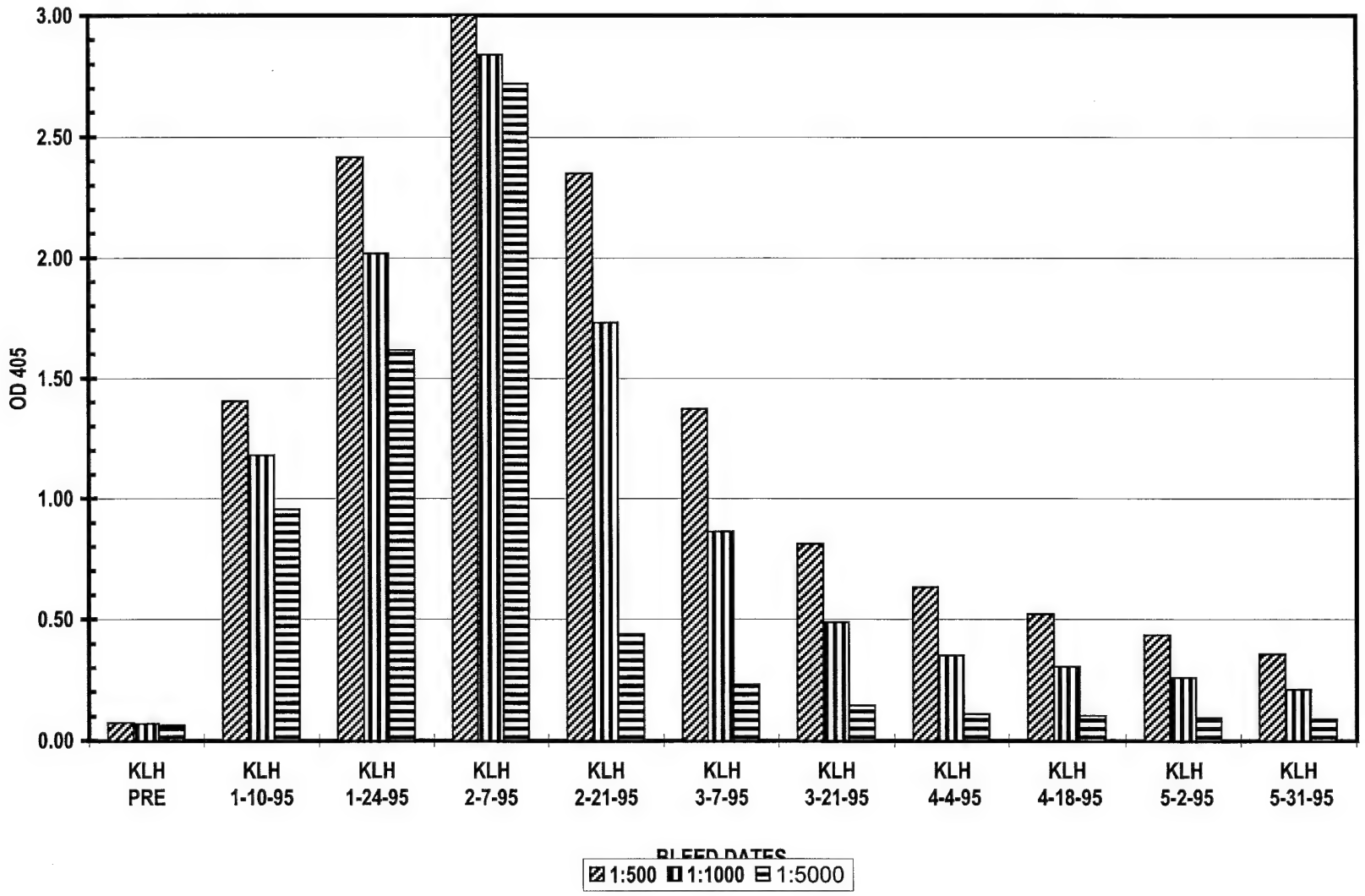
| **2/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **5.41** | **10.71** | **3.68** | 0.64 |
| tetanus toxoid | 1.04 | 1.06 | 1.06 | 0.61 |
| gp130 | XXX | **2.55** | 0.98 | 0.64 |

| **3/21** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | **9.92** | **7.50** | **7.06** | 1.42 |
| tetanus toxoid | **3.14** | **2.49** | **2.28** | 1.80 |
| gp130 | 0.45 | **2.34** | 0.71 | 1.24 |

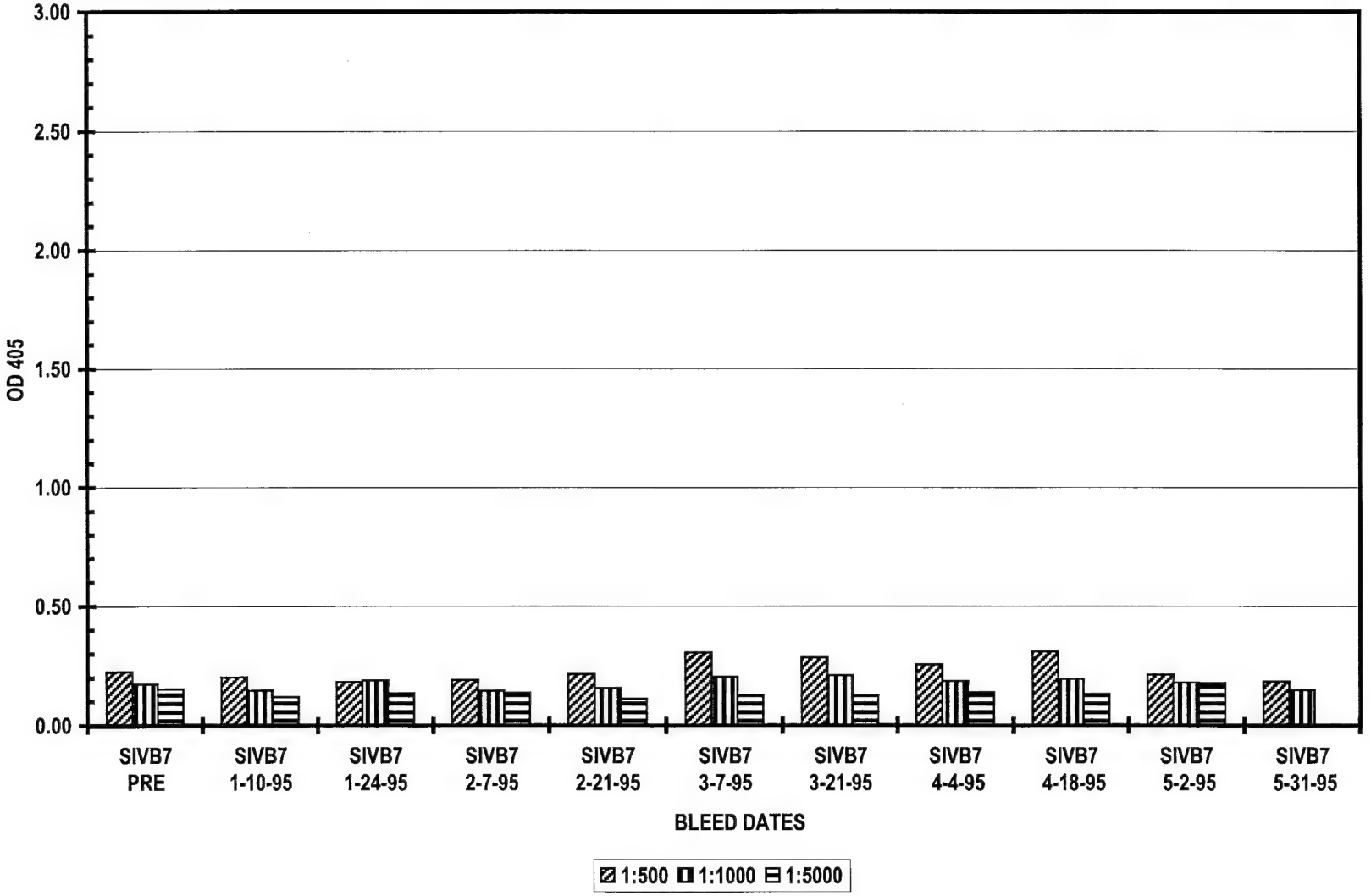
| **4/18** ug/mL: | 10.0 SI | 3.3 SI | 1.0 SI | 0.1 SI |
|---|---|---|---|---|
| KLH | 1.05 | 0.90 | 0.69 | 0.74 |
| tetanus toxoid | 1.85 | 1.53 | 1.75 | 1.22 |
| gp130 | 0.55 | 0.56 | 0.72 | 0.94 |

Prepared by Dave Nabi 8/25/95

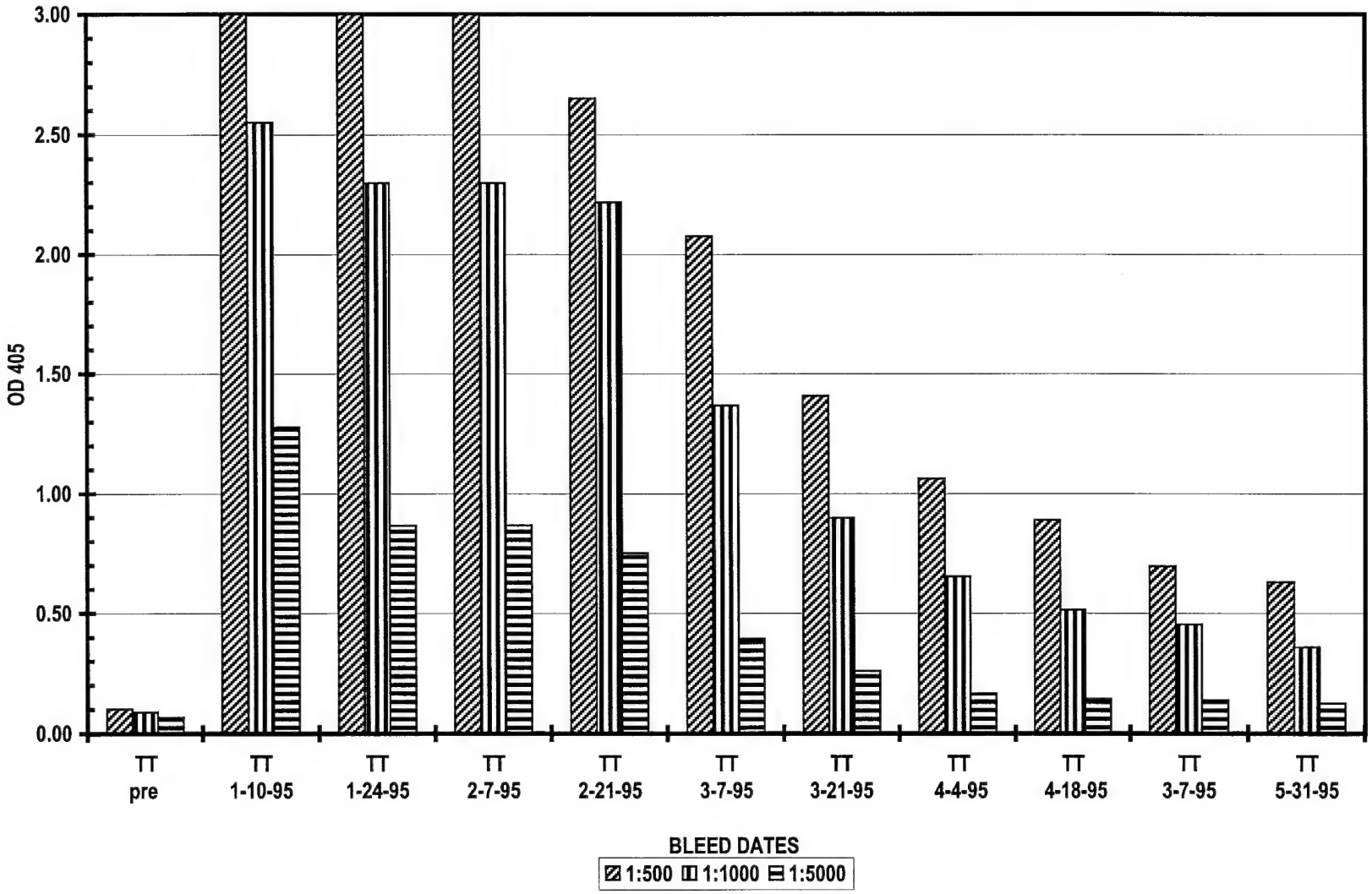
# AB PRODUCTION OF MONKEY#26161 USING KLH AS ANTIGEN



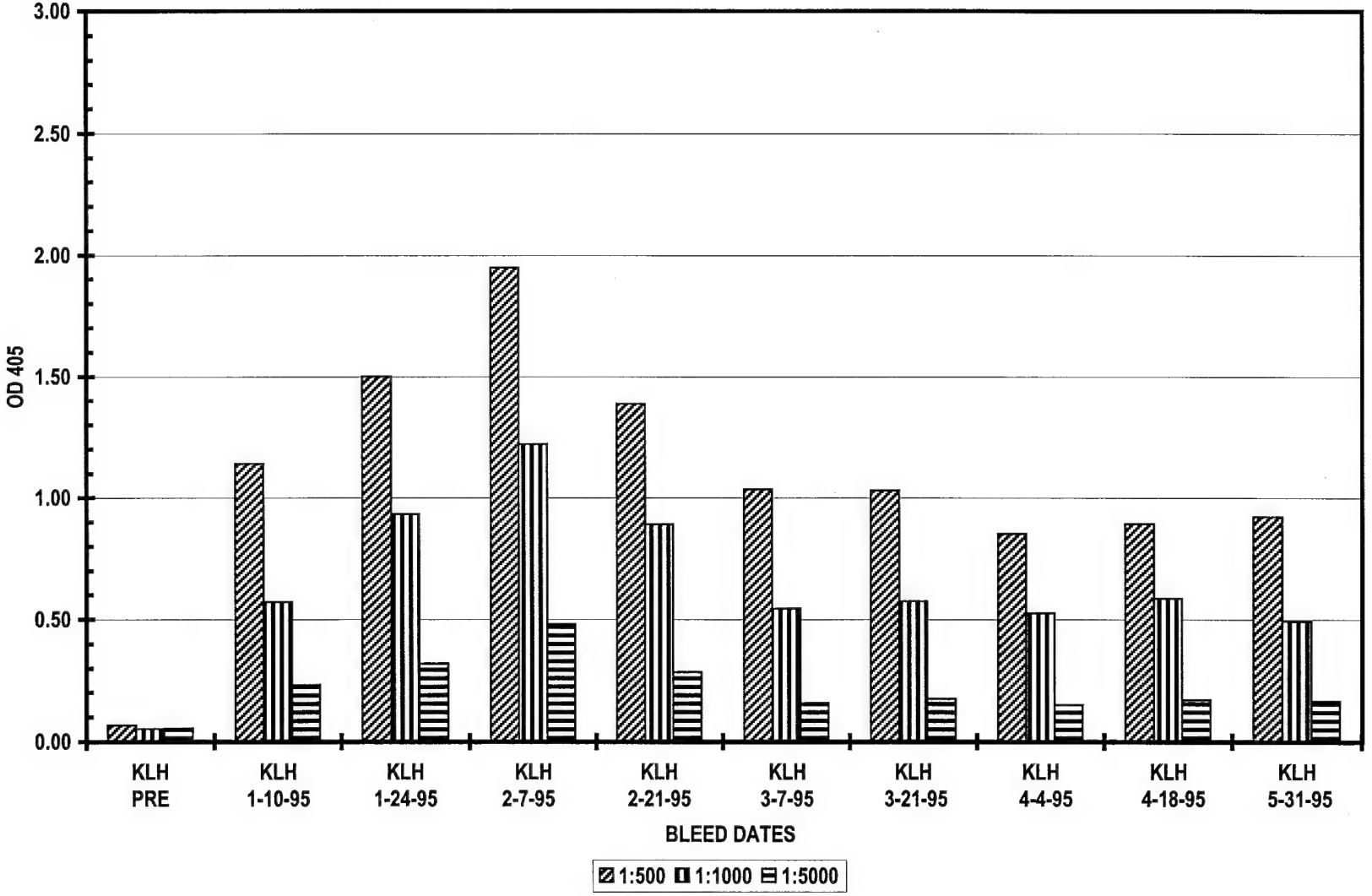
# AB PRODUCTION OF MONKEY#26161 USING SIVB7 AS ANTIGEN



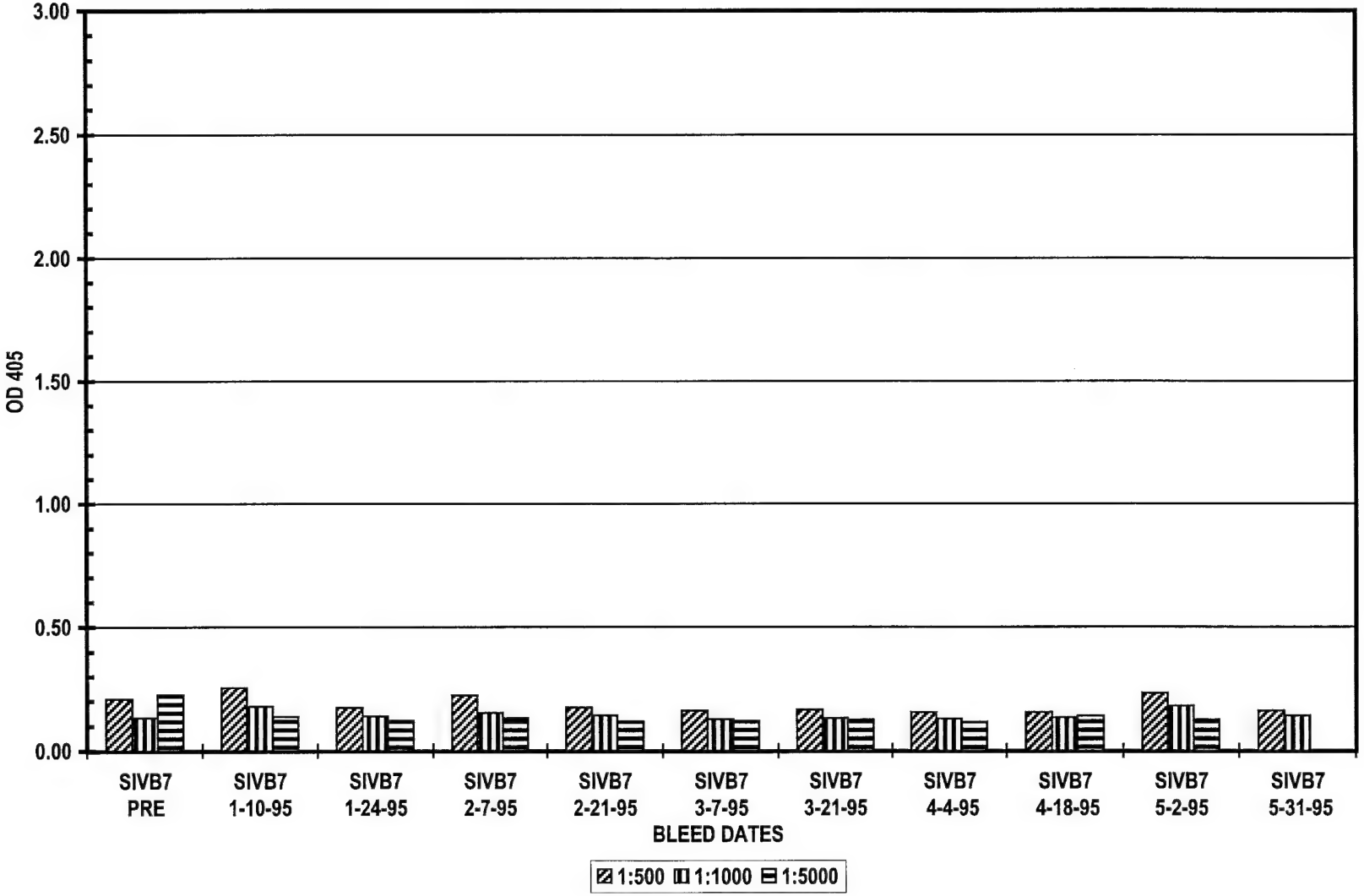
## AB PRODUCTION OF MONKEY#26161 USING TETANUS TOXOID AS ANTIGEN



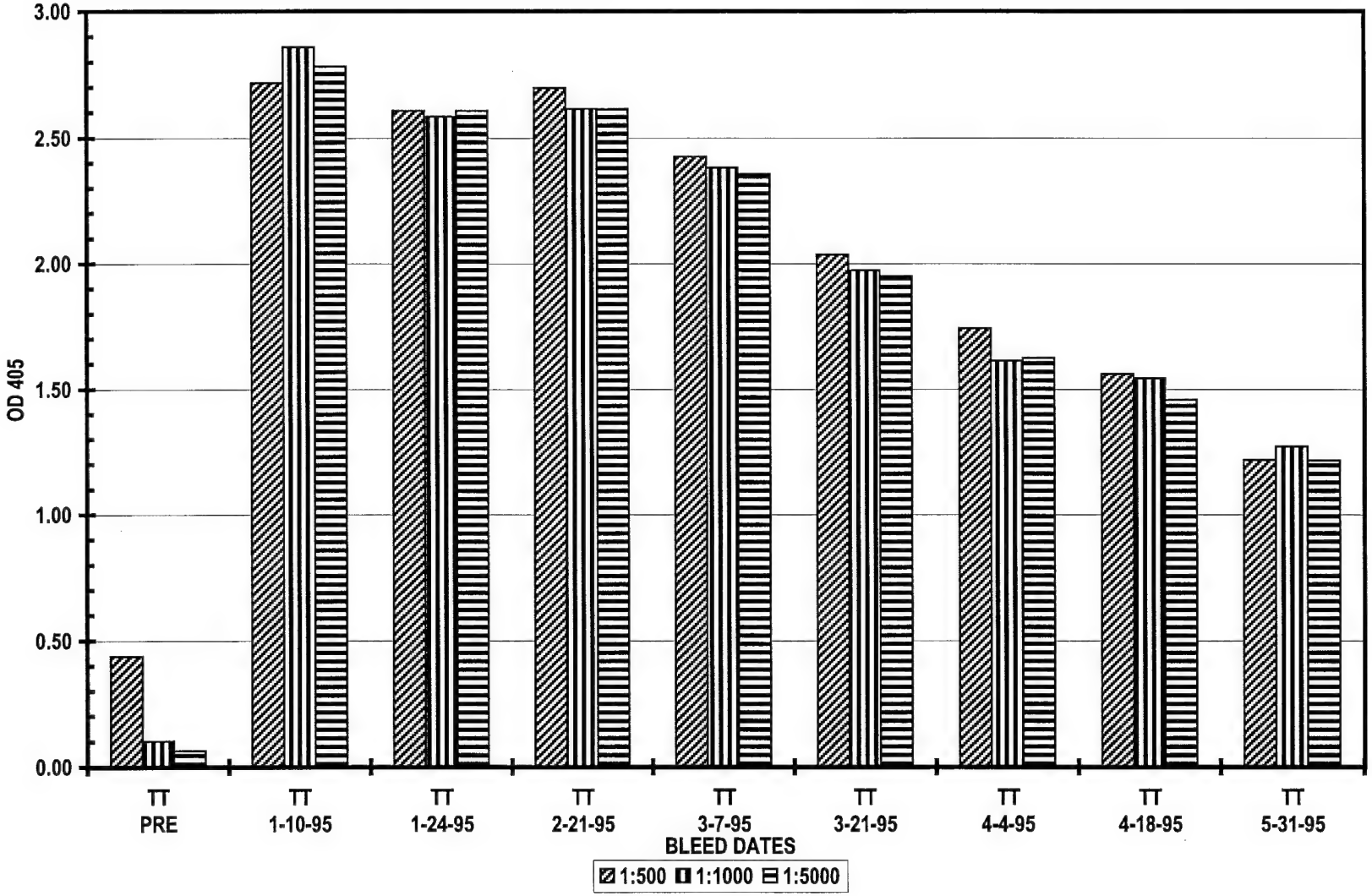
## AB PRODUCTION OF MONKEY #26313 USING KLH AS ANTIGEN
## (not challenged)



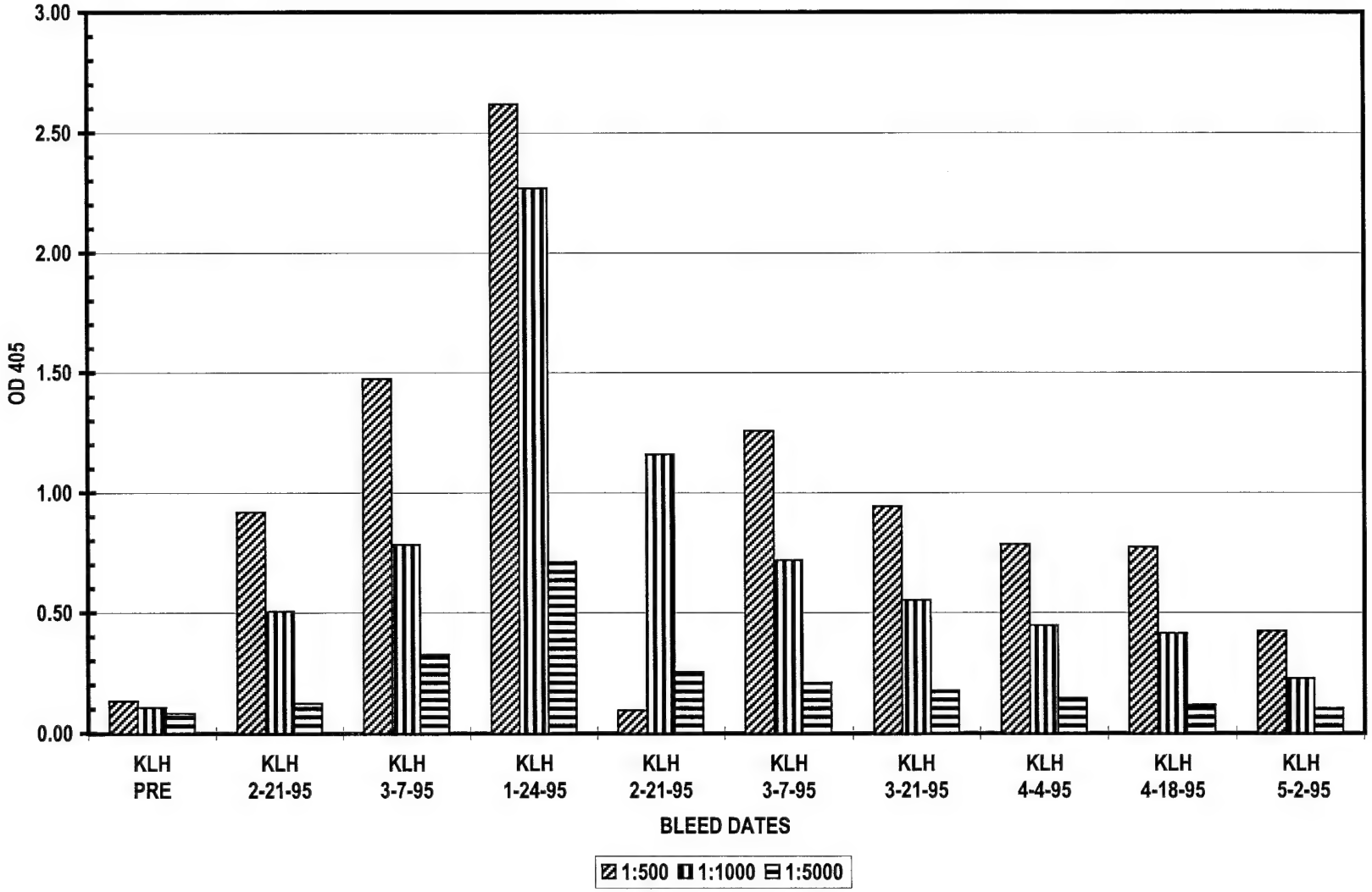
## AB PRODUCTION OF MONKEY#26313 USING SIVB7 AS ANTIGEN
## (not challenged)



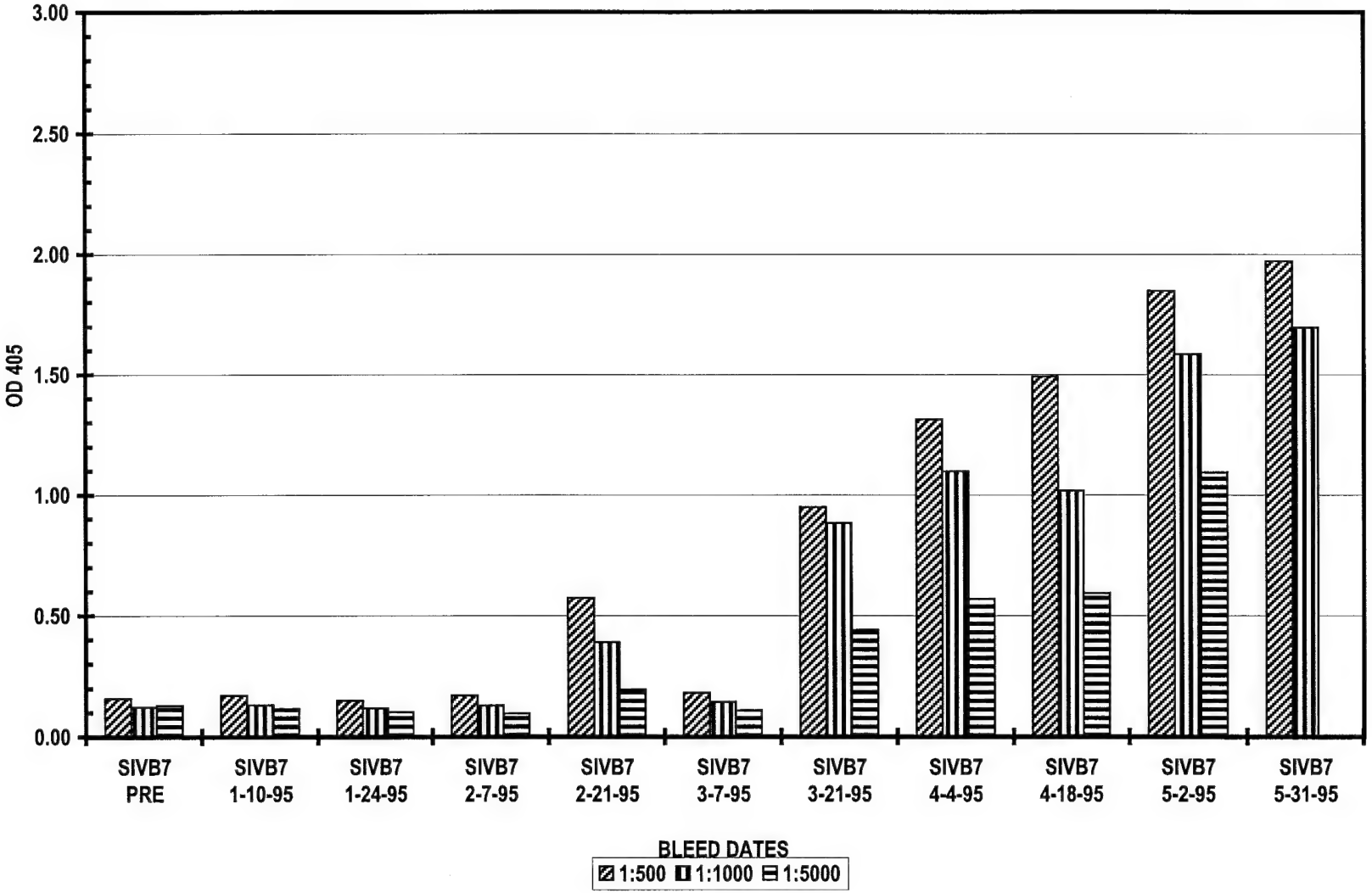
## AB PRODUCTION OF MONKEY#26313 USING TETANUS TOXOID AS ANTIGEN
## (not challenged)



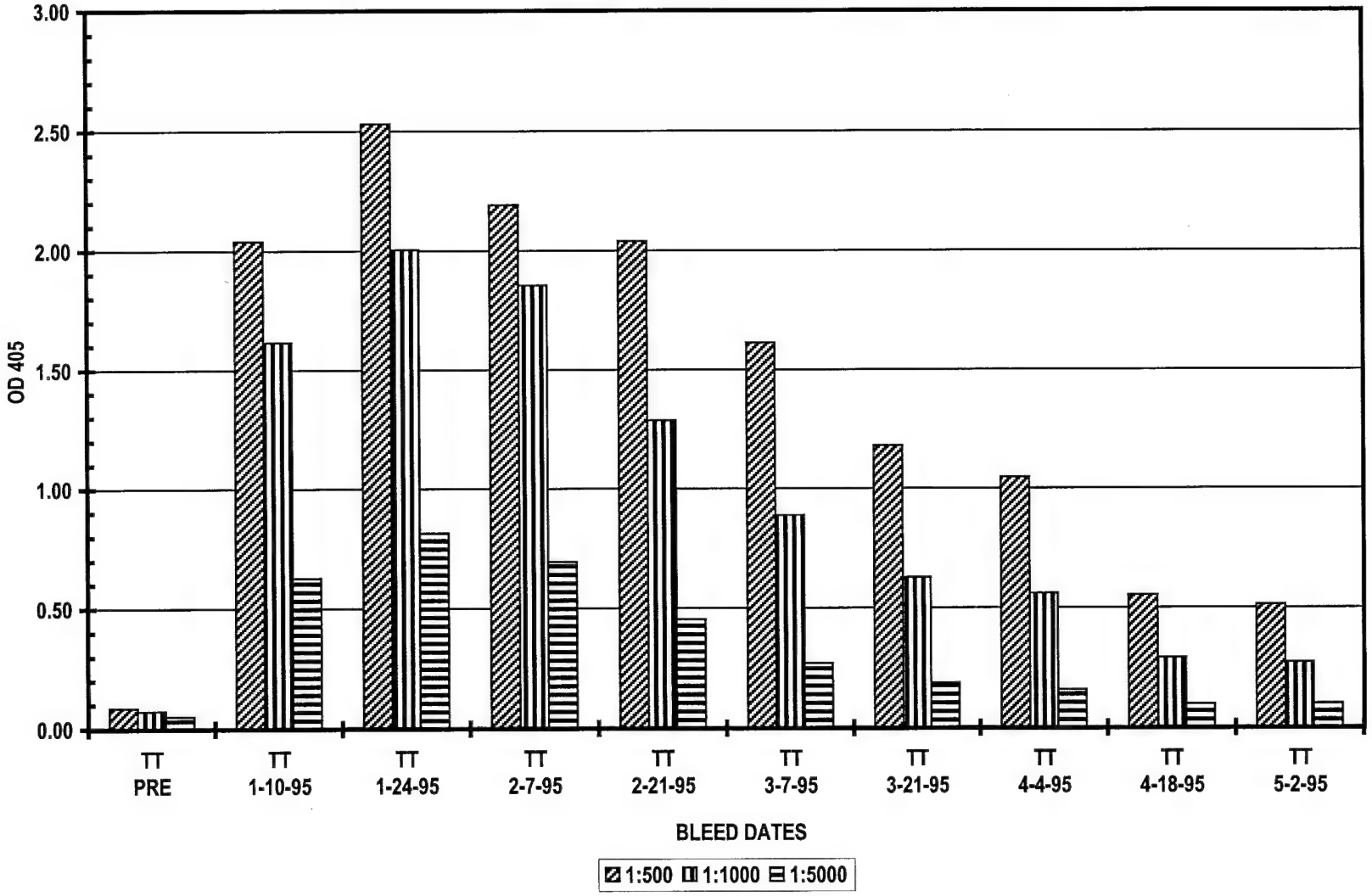
## AB PRODUCTION OF MONKEY#26320 USING KLH AS ANTIGEN



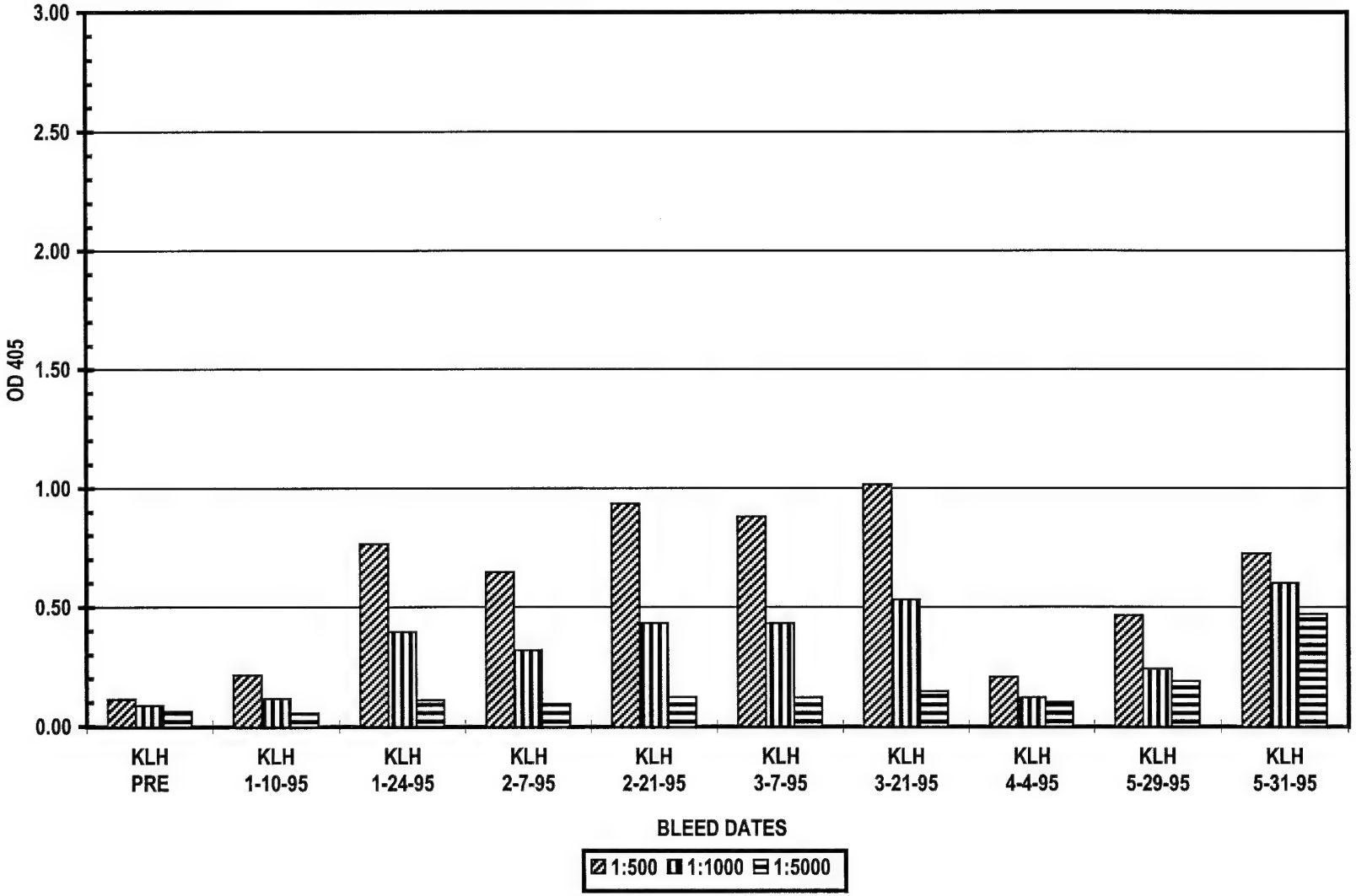
# AB PRODUCTION OF MONKEY #26320 USING SIVB7 AS ANTIGEN



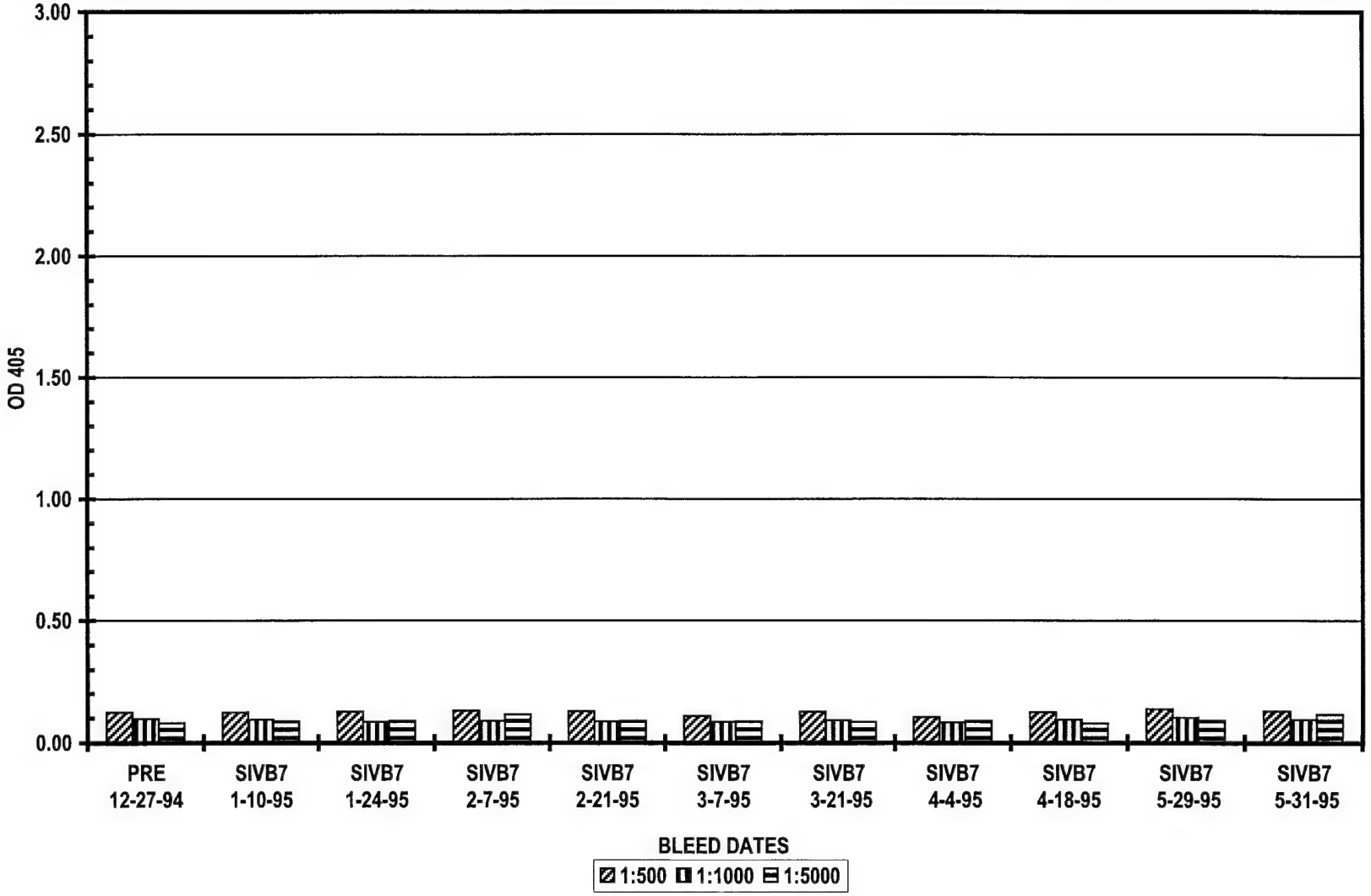
## AB PRODUCTION OF MONKEY#26320 USING TETANUS TOXOID AS ANTIGEN



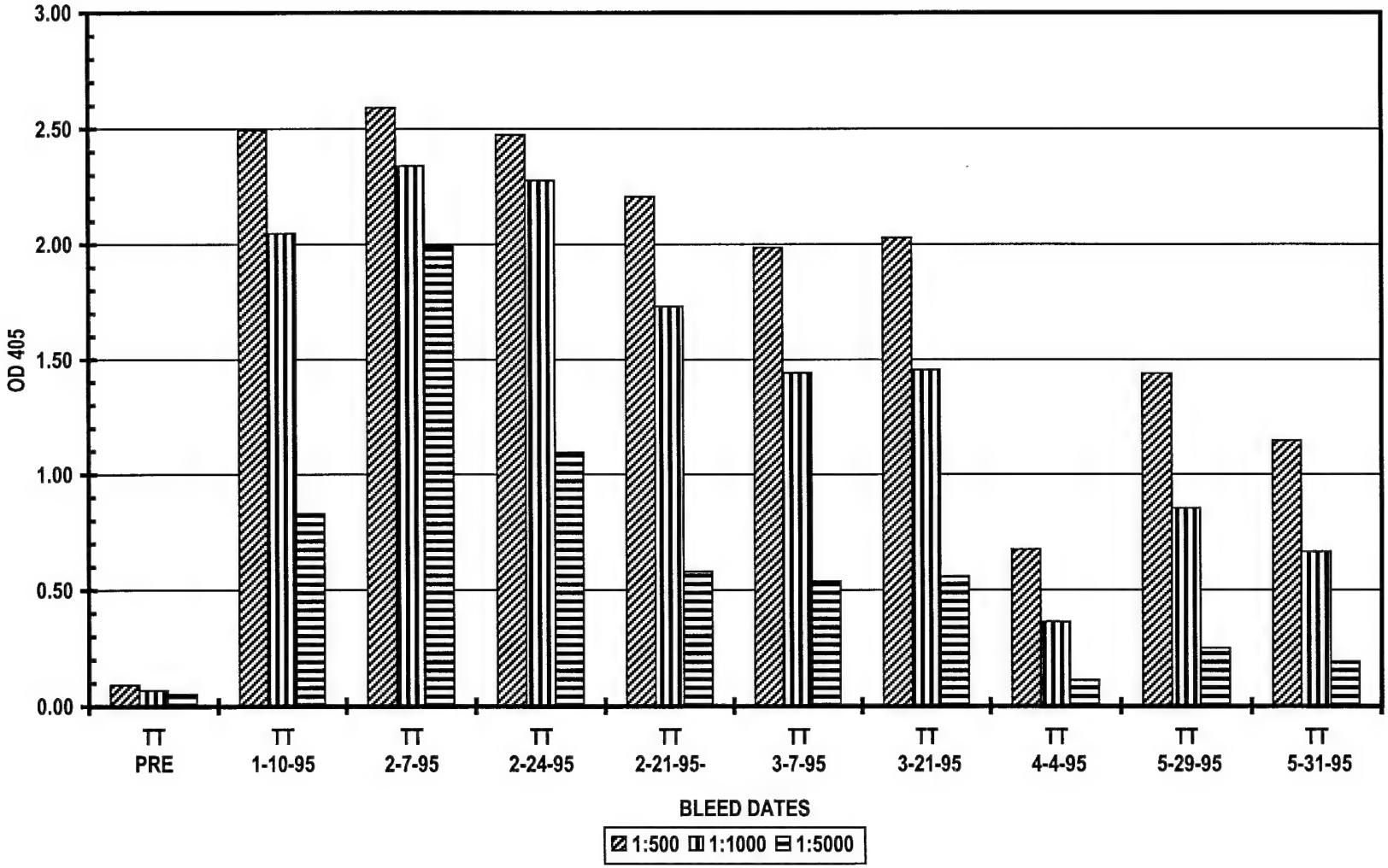
## AB PRODUCTION OF MONKEY#26438 USING KLH AS ANTIGEN
## (not challenged)



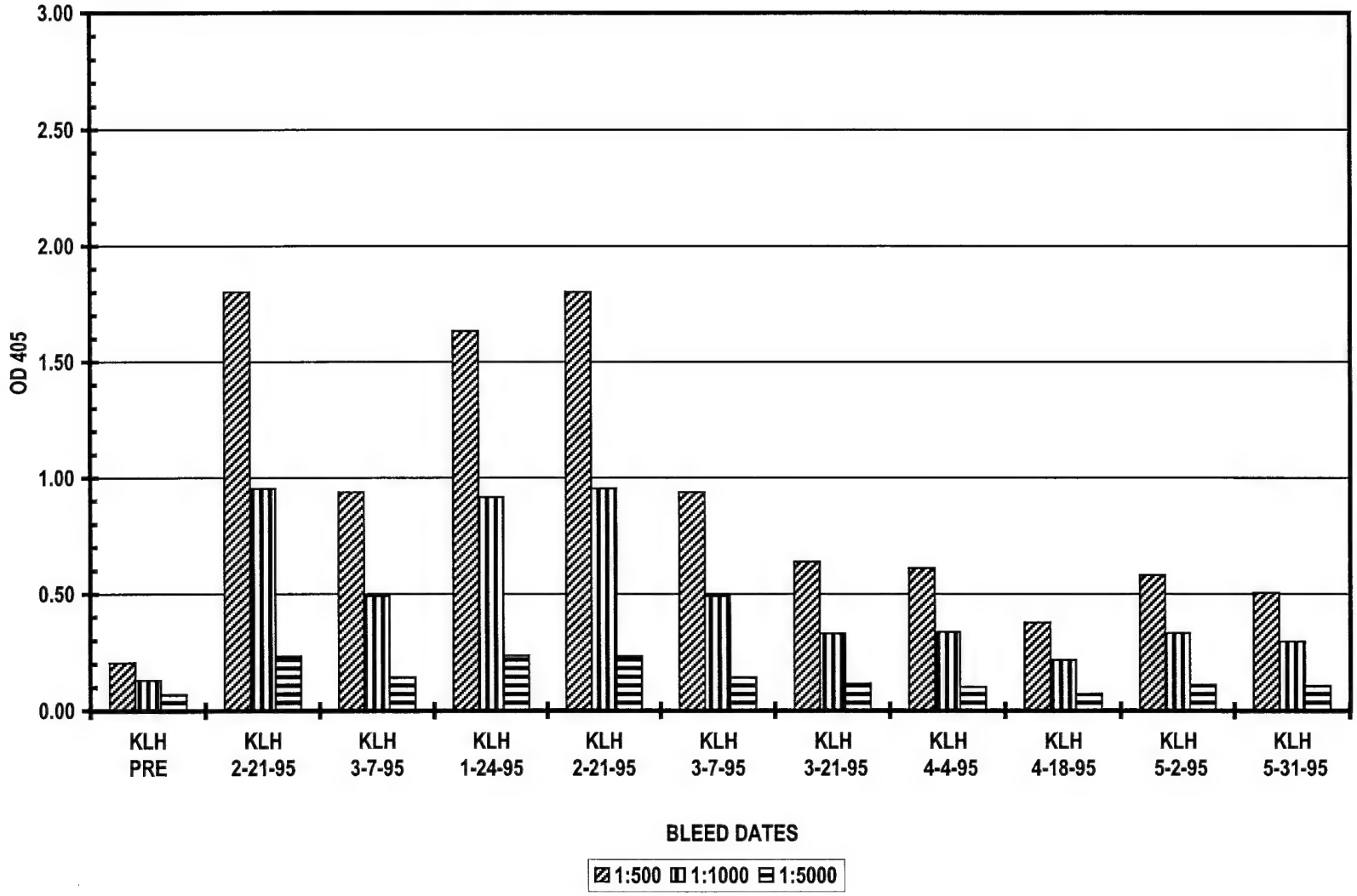
## AB PRODUCTION OF MONKEY#26438 USING SIVB7 AS ANTIGEN
## (not challenged)



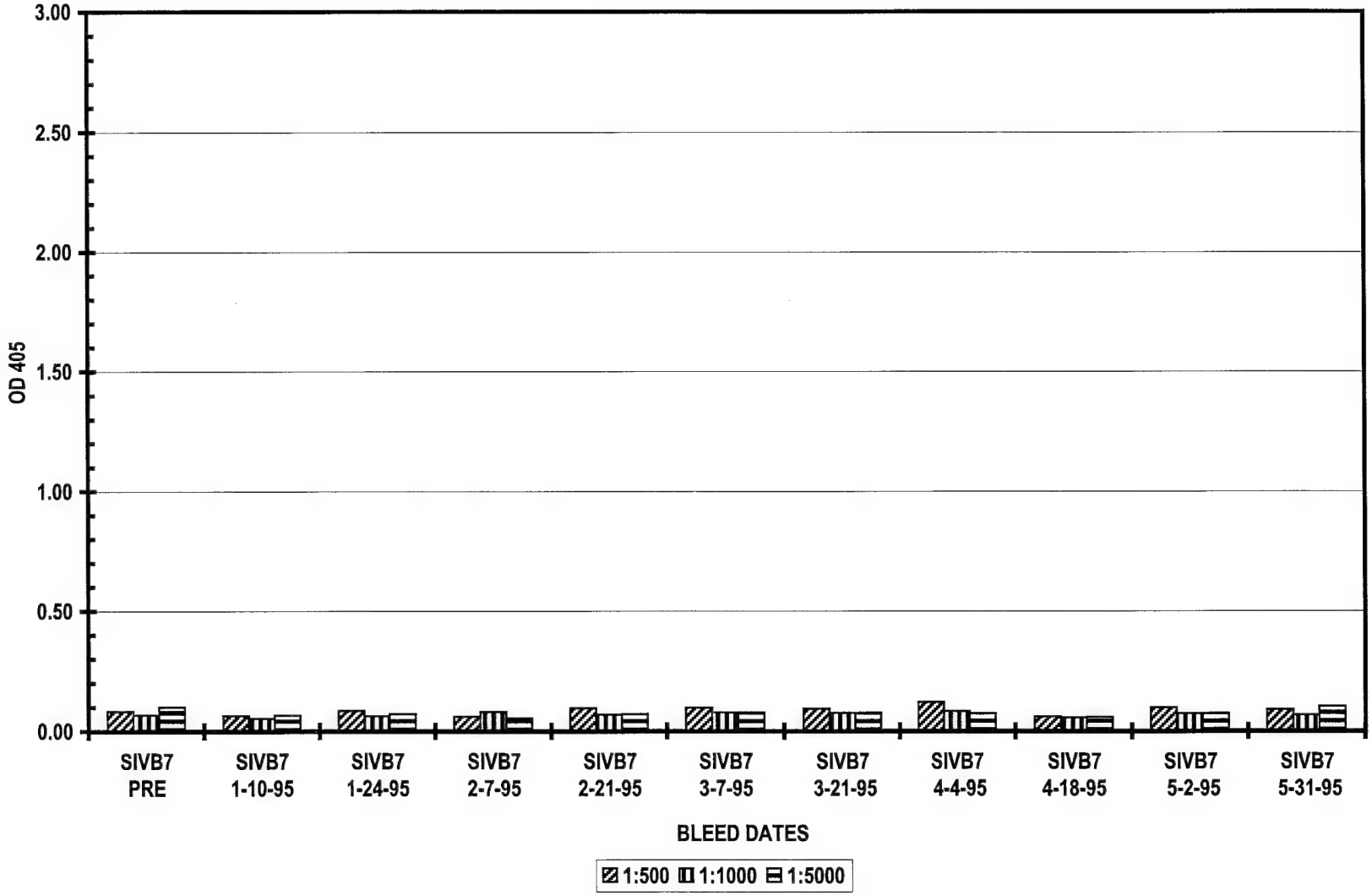
## AB PRODUCTION OF MONKEY#26438 USING TETANUS TOXOID AS ANTIGEN
## (not challenged)



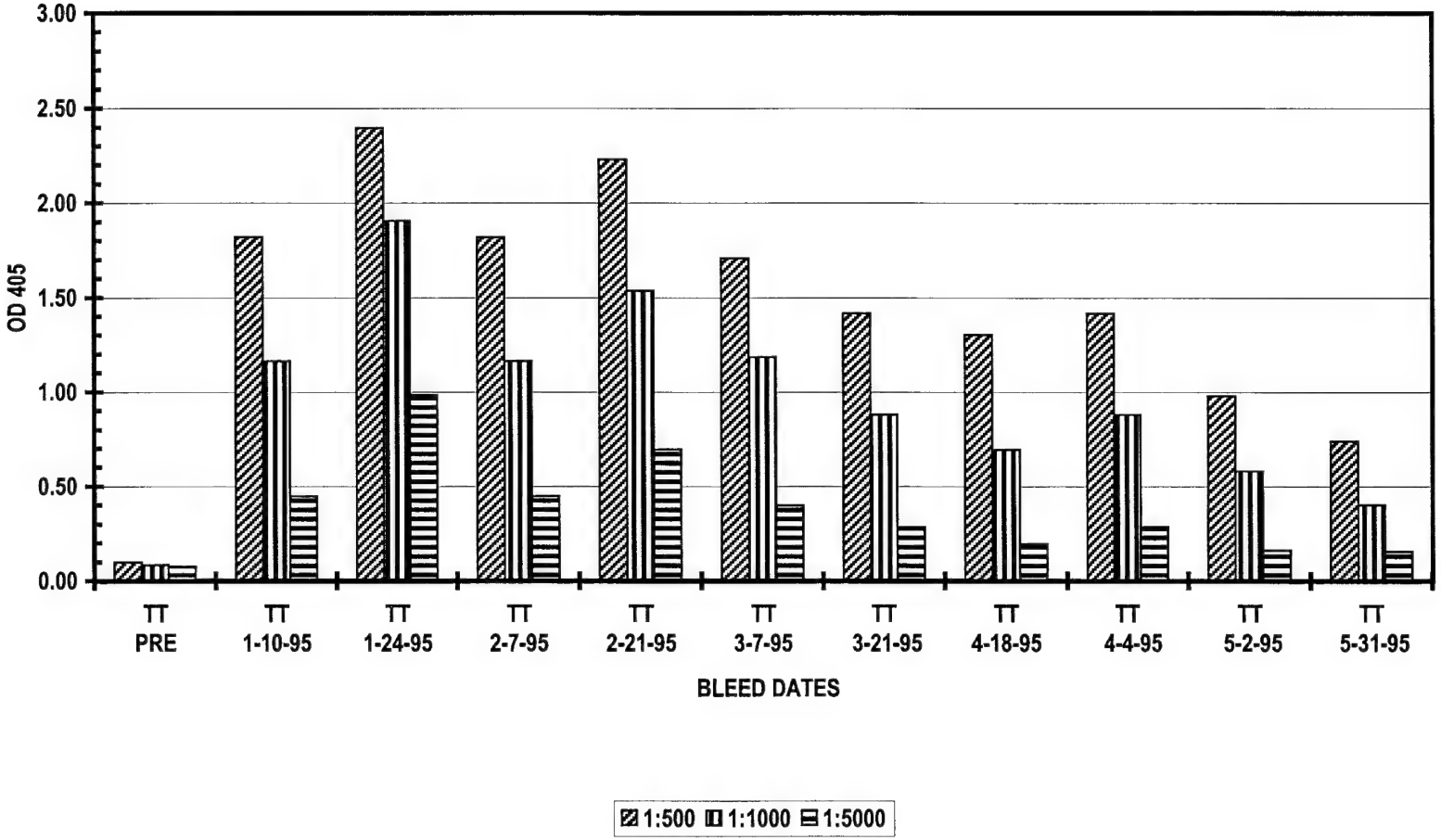
# AB PRODUCTION OF MONKEY#26470 USING KLH AS ANTIGEN



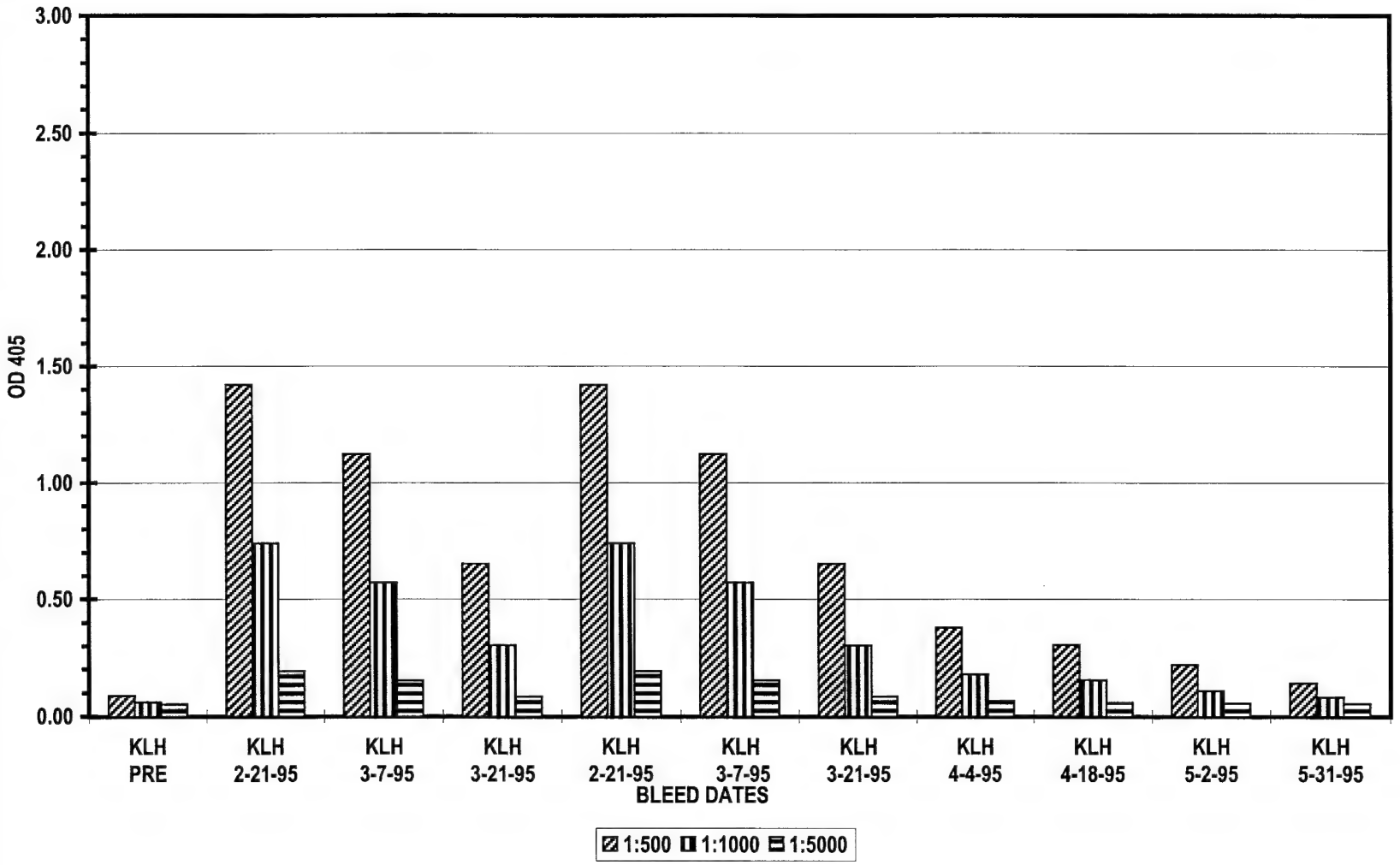
# AB PRODUCTION OF MONKEY#26470 USING SIVB7 AS ANTIGEN

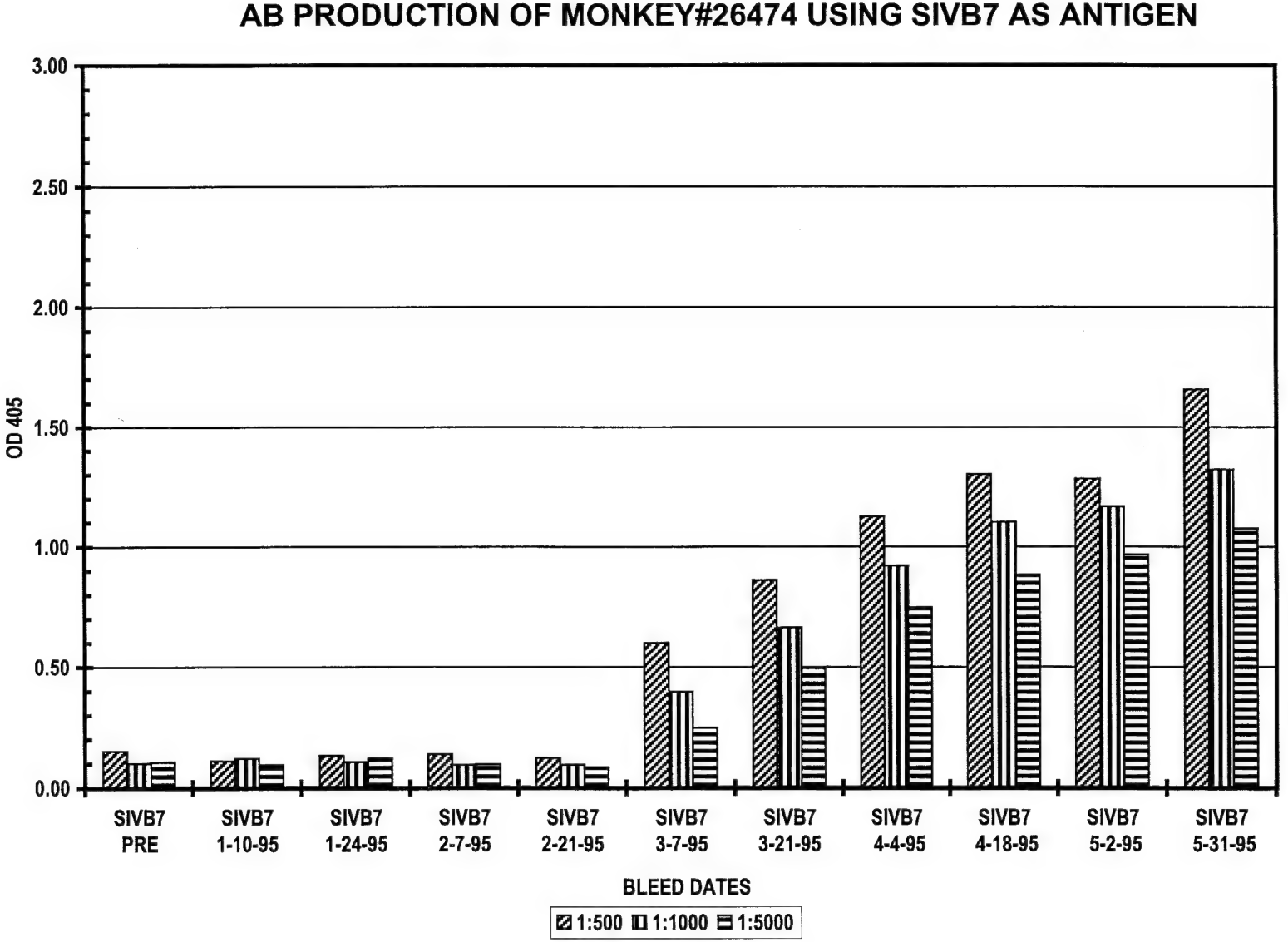


# AB PRODUCTION OF MONKEY#26470 USING TETANUS TOXOID AS ANTIGEN

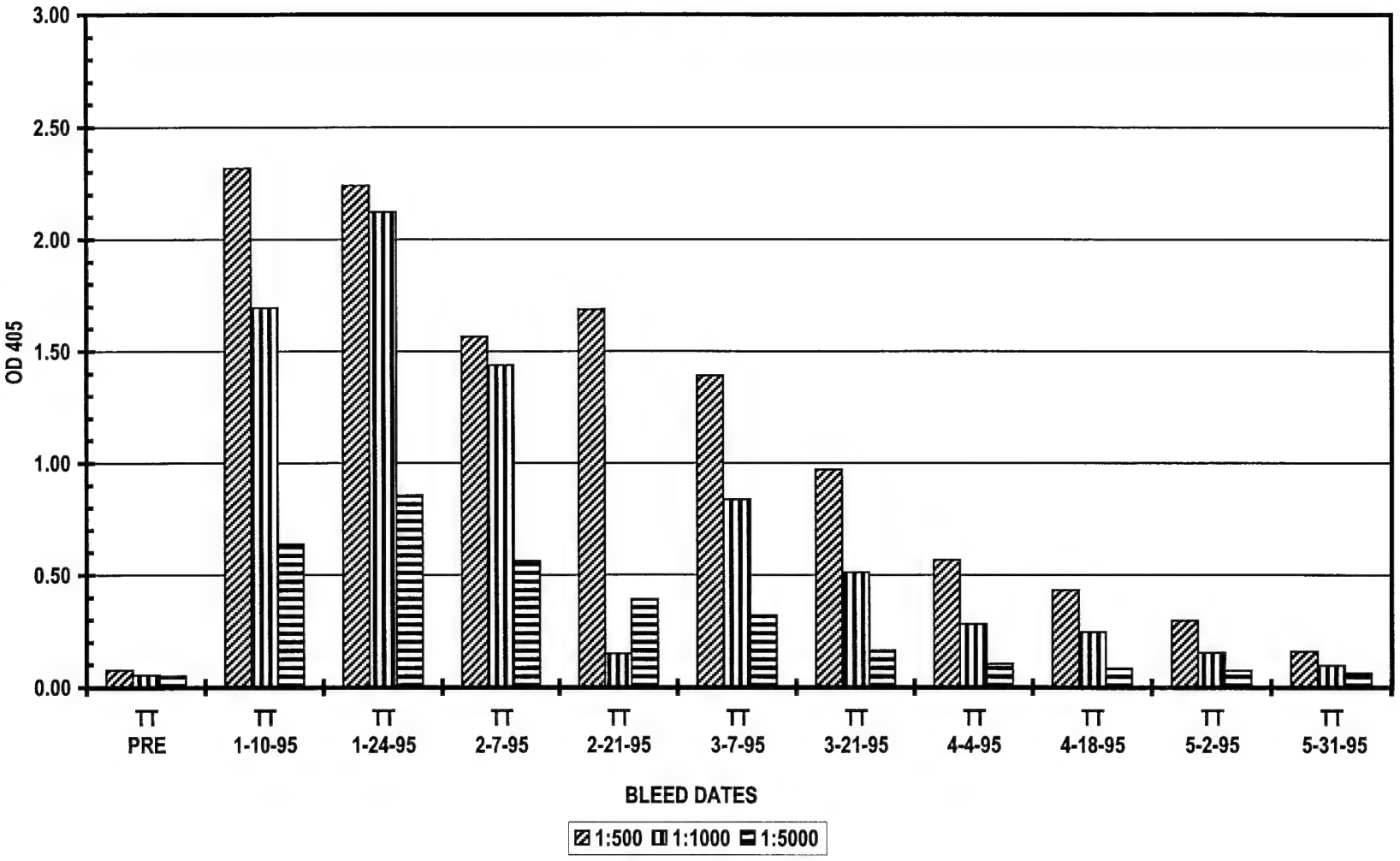


# AB PRODUCTION OF MONKEY#26474 USING KLH AS ANTIGEN




AB PRODUCTION OF MONKEY#26474 USING SIVB7 AS ANTIGEN
3.00
2.50
2.00
1.50
1.00
0.50
0.00
OD 405
SIVB7 PRE
SIVB7 1-10-95
SIVB7 1-24-95
SIVB7 2-7-95
SIVB7 2-21-95
SIVB7 3-7-95
SIVB7 3-21-95
SIVB7 4-4-95
SIVB7 4-18-95
SIVB7 5-2-95
SIVB7 5-31-95
BLEED DATES
1:500
1:1000
1:5000

# AB PRODUCTION OF MONKEY#26474 USING TETANUS TOXOID AS ANTIGEN



# Appendix 3:
# Revised budget, Emory Subcontract

DD

Principal Investigator/Program Director (Last, first, middle): _____

**DETAILED COST ESTIMATE - PROJECT PERIOD 2**

**EMORY UNIVERSITY**

FROM 10/1/95 THROUGH 9/30/96

| PERSONNEL (Applicant Organization Only) | | | | | DOLLAR AMOUNT REQUESTED (omit cents) | | |
|---|---|---|---|---|---|---|---|
| NAME | ROLE | APPT | % EFF | BASE SALARY | SAL REQST | FRINGE BEN | TOTALS |
| Dr, A. A. Ansari | Prin Invst | 12 | 7.5 | $124,808 | $9,361 | $2,340 | $11,701 |
| Dr. F. Villinger | Co-Invest | 12 | 30 | $41,800 | $12,540 | $3,135 | $15,675 |
| Sukhdev Brar | Res. Tech II | 12 | 75 | $28,528 | $21,396 | $5,349 | $26,745 |
| | | | | | $0 | $0 | $0 |
| | | | | | $0 | $0 | $0 |
| | | | | | $0 | $0 | $0 |
| | | | | | $0 | $0 | $0 |
| | | | | | | | |
| | | | | | | | |
| | | | SUBTOTALS | | $43,297 | $10,824 | $54,121 |

| | | | |
|---|---|---|---|
| CONSULTANT COSTS | | | $0 |
| EQUIPMENT (Itemize) | | | |
| SUPPLIES (Itemize by category) | | | |
| Oligos & RT PCR | $15,000 | | |
| Tissue culture | $2,381 | | |
| ELISA kits ($500/kit) | $10,000 | | |
| Bioassay supplies | $4,090 | In situ hybridization $13,000 | $44,471 |
| TRAVEL | | | |
| PATIENT CARE COSTS | INPATIENT | | $0 |
| | OUTPATIENT | | $0 |
| ALTERATIONS AND RENOVATIONS (Itemize by category) | | | $0 |
| OTHER EXPENSES (Itemize by category) | | | |
| **TOTAL DIRECT COSTS** | | | **$98,592** |
| **TOTAL INDIRECT COSTS** | 57% OF DIRECT COSTS | | **$56,197** |
| **TOTAL COSTS FOR SECOND BUDGET PERIOD** | | | **$154,789** |

# Appendix 4:
# Memorandum from California Primate Research Center

## CALIFORNIA PRIMATE RESEARCH CENTER - Cost Estimate for Proposed Research Grant

Proposed Project: Optimization of Vaccination Conditions in Primates
Prepared for: Dr. Gary Rhodes
Prepared by: Jenny Short, Research Services

Date: 9/5/95 Year 1 Start date: 1/1/96

| A. Animal Costs. | No. of Animals | No. of Days | Housing Type | Rate / Day | Total Cost | | Comments |
|---|---|---|---|---|---|---|---|
| per diem | 9 | 120 | Indoor | 3.45 | 3,726.00 | | |
| Total Animal Costs | | | | | | $3,726.00 | |

| B. Labor Costs. | Hours / Animal | Hourly Rate | Total Animals | Number | Total Cost | | Comments |
|---|---|---|---|---|---|---|---|
| SRA | 5.00 | 24.06 | | 4 | 481.20 | | workorders, scheduling, proje |
| SRA | 0.50 | 24.06 | 9 | 1 | 108.27 | | baseline bleed and vaccinate |
| SRA | 0.25 | 24.06 | 9 | 4 | 216.54 | | bloods; 3, 6,9,12 weeks |
| Animal tech | 0.25 | 21.60 | 9 | 4 | 194.40 | | weights |
| Veterinarian | 1.00 | 37.64 | 9 | | 338.76 | | general clinical and project su |
| Total Labor Costs | | | | | | $1,339.17 | |

C. Miscellaneous Costs.

| Supplies | Quantity | cost/item | Total | |
|---|---|---|---|---|
| blood sample supplies | 45 | 2.50 | 112.50 | |
| CBC | 18 | 18.00 | 324.00 | |
| misc. animal handling supplies | | 50.00 | 50.00 | |
| Total Misc. Costs | | | | $486.50 |

| | | |
|---|---|---|
| D. Total Project Costs: | | $5,551.67 |
| Indirect Cost @ 18.9% | | 1,049.27 |
| **Total Study Cost** | | **7,087.44** |